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# On the Plasminogen Activator Activity in Human Veins and Arteries

by

Harald Ljungné<sup>r</sup>  
          



Malmö 1982





ON THE PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN  
VEINS AND ARTERIES

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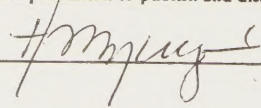
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| <b>Title and subtitle</b><br>On the plasminogen activator activity in human veins and arteries                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                               |                            |
| <b>Abstract</b><br><p>In this study the PA activity was determined in various superficial, deep veins and in arteries in healthy subjects and in patients with various diseases. The correlations in the activity between various veins and between arteries and concomitant veins were analysed. The pre-, per, and postoperative PA activity in superficial hand veins was studied in relation to major surgery and in relation to two different types of prostatic surgery. The PA activity was also assessed in patients with risk factors known to predispose to postoperative deep venous thrombosis (DVT) and in a subgroup of patients with verified postoperative DVT. The response in the fibrinolytic system and in factor VIII was analysed in patients treated with two different mechanical thromboprophylactic methods.</p> <p>The material consisted of healthy subjects, patients with diabetes mellitus, terminal uraemia and arteriosclerosis. Biopsy specimens were obtained in most cases in association with surgery.</p> <p>The PA activity was semiquantified with a modification of Todd's histochemical technique.</p> <p>There was a correlation in the PA activity between superficial and deep leg veins, between superficial hand and foot veins and between various arteries and concomitant veins. There was a significantly postoperative decrease in PA activity in patients subjected to major surgery. An abnormally low postoperative PA activity was a common finding in patients undergoing major surgery. Nine out of 10 patients with postoperative DVT had an abnormally low postoperative PA activity. Obesity and a duration of the operation of 180 min or more were two factors which influenced the frequency of patients with a postoperative PA decrease. Neither of the two mechanical thromboprophylactic methods were accompanied by any alterations in the fibrinolytic system nor in factor VIII.</p> |                                               |                            |
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## E R R A T A

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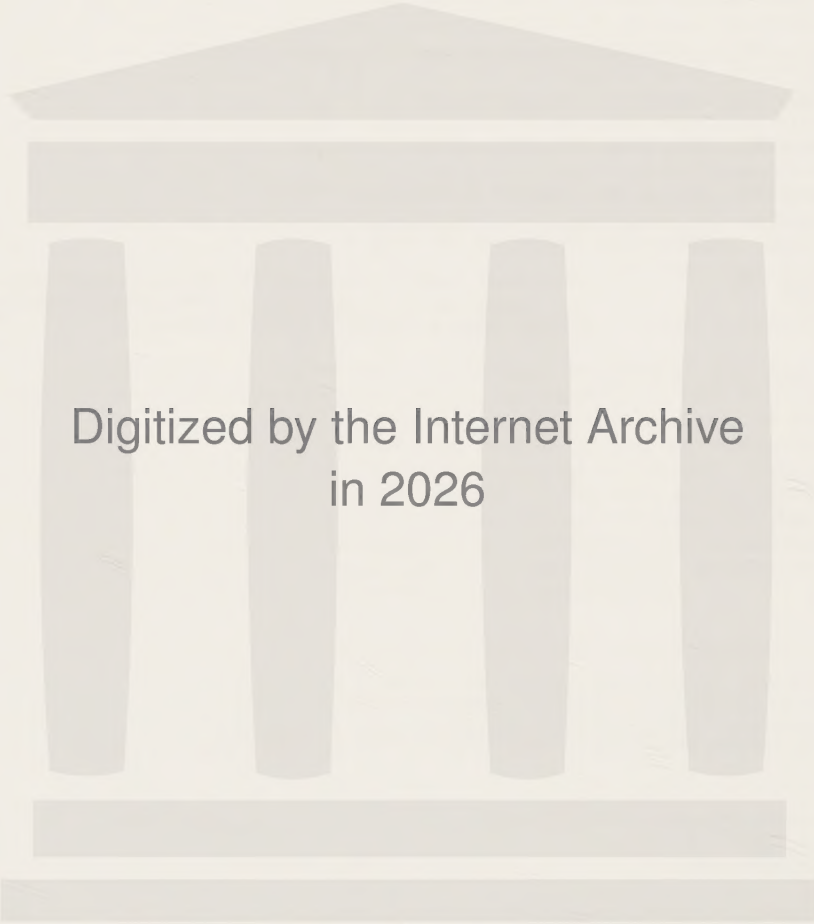
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- I      Comparison between the plasminogen activator activity in  
         walls of superficial, muscle and deep veins  
         Ljungnér H, Bergqvist D, Isacson S, Nilsson IM  
         Thromb Res 22:295-302, 1981
  
- II     Comparison of the plasminogen activator activity of super-  
         ficial hand and foot veins  
         Ljungnér H, Bergqvist D  
         Thromb Res 1982 (in press)
  
- III    The influence of major surgery on plasminogen activator  
         activity in superficial hand veins  
         Ljungnér H, Bergqvist D, von Hebel I, Isacson S  
         Eur Surg Res 1982 (in press)
  
- IV     Plasminogen activator activity in patients undergoing  
         transvesical and transurethral prostatectomy  
         Ljungnér H, Bergqvist D, Isacson S  
         Eur Urol 1982 (in press)
  
- V      Effect of intermittent pneumatic and graduated static  
         compression on factor VIII and the fibrinolytic system  
         Ljungnér H, Bergqvist D, Nilsson IM  
         Acta Chir Scand 147:657-664, 1981
  
- VI     Comparison of the plasminogen activator activity in  
         biopsy specimens of human arteries and veins  
         Ljungnér H, Bergqvist D  
         Thromb Haemost (submitted)

The articles will be referred to in the text by the Roman numerals above.

## CONTENTS

|                                               |    |
|-----------------------------------------------|----|
| INTRODUCTION .....                            | 5  |
| GENERAL BACKGROUND .....                      | 6  |
| BACKGROUND TO THE PRESENT INVESTIGATION ..... | 20 |
| PURPOSE .....                                 | 23 |
| MATERIAL AND METHODS .....                    | 24 |
| RESULTS .....                                 | 30 |
| DISCUSSION .....                              | 34 |
| CONCLUSIONS .....                             | 53 |
| REFERENCES .....                              | 55 |

## INTRODUCTION

Deep venous thrombosis (DVT) is a common finding in patients after major surgery, the main immediate risk being fatal pulmonary embolism. The etiology of postoperative DVT is multifactorial, and according to Virchow's triad, changes in the blood flow, in the blood components and in the vessel wall are of pathogenetic importance. The attention has been focused on the role played by venous stasis and changes in the coagulation system in the development of postoperative venous thrombosis, but the significance of the fibrinolytic system has received less attention, especially that of the plasminogen activators (PA) in the vessel wall. On the other hand patients with recurrent venous thrombosis often have an impaired fibrinolytic activity in the vessel wall and/or in plasma (Isacson & Nilsson 1972).

An intact fibrinolytic system is of importance in several aspects. Thus, it has been suggested that there is normally a dynamic equilibrium between fibrin deposition and fibrinolysis and that disturbances of this balance may result in fibrin deposition, thrombus formation and atherosclerosis (reviewed by Astrup 1978). Astrup (1968) have shed light upon the fibrinolytic system in the process of tissue repair. Physiological thrombolysis and recanalization of established thrombi is dependent on the fibrinolytic system (Kwaan & Astrup 1965, Pandolfi et al 1969).

The main aim of this study was to assess the PA activity in surgical patients, and being a surgeon I had the possibility to obtain biopsy specimens of different deep veins and arteries in normal subjects and in patients with various diseases subjected to surgery.



## GENERAL BACKGROUND

### Historical remarks

In 1893 Dastre coined the term fibrinolysis to designate the breakdown of a fibrin clot. Later, in 1906, Morawitz noted not only that the blood from victims of sudden death lacked fibrinogen but also that it could destroy the fibrinogen and fibrin of normal blood. In 1907 Opies and Barker found that addition of chloroform to the blood induced fibrinolytic activity, which was localized to the globulin fraction.

Tissue fibrinolytic activity was first described by Fleisher & Loeb (1915) and Fischer (1930) observed that in vitro cultures of normal and neoplastic cells were fibrinolytically active.

Investigations by Kaplan (1944) and Christensen (1945) revealed that fibrinolysis was due to an enzyme precursor which was converted to an active enzyme. The precursor was designated plasminogen and the enzyme plasmin (Christensen & MacLeod 1945). At the same time Astrup & Permin (1947) showed that the fibrinolytic activity of tissues was due to an activator of plasminogen (tissue activator).

### The fibrinolytic system

There are four main components of the fibrinolytic system, i.e. plasminogen, plasmin, activators and inhibitors (Fig. 1).

The conversion of plasminogen to plasmin is brought about by various activators localized in tissues (Astrup & Permin 1947, Astrup.1969), in the blood (Biggs et al 1947, Müllertz 1953, Fearnley 1965, Nilsson et al 1970) and in the urine (Macfarlane & Pilling 1947, Williams 1951, Kjeldgaard & Plough 1957). The activator in the urine was named urokinase (Sobel et al 1952). Activators occur also in the cerebrospinal fluid, bile, tears, seminal plasma and saliva (Albrechtsen 1959).

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graph TD
    Streptokinase --> Plasminogen
    SK-plasminogen[SK-plasminogen complex] --> Plasminogen
    SK-plasmin[SK-plasmin complex] --> Plasminogen
    EACA --> Plasminogen
    AMCA --> Plasminogen
    Activators[Blood activators  
Tissue activators  
Urokinase] --> Plasminogen
    Inhibitors1[INHIBITORS of the plasminogen activation  
"Urokinase inhibitors"] --> Plasminogen
    Plasminogen --> Plasmin
    Inhibitors2[INHIBITORS  
α2-macroglobulin  
α2-antiplasmin] --> Plasmin
    Plasmin --> Fibrinogen
    Fibrinogen --> Fibrin
    Fibrin --> X-product
    Fibrin --> Y-product
    Fibrin --> E-product
    Fibrin --> D-product
    X-product --> D-product
    Y-product --> D-product
    Fibrin -.->|Inhibition of fibrin polymerisation| Fibrinogen
    Fibrin -.->|Antithrombin effect| Fibrinogen
  
```

Plasminogen activation can be induced in the blood by contact activation of coagulation factor XII (Hageman factor) (Niewiarowski & Prou-Wartelle 1959). Activation of the fibrinolytic system via this pathway requires the interaction with at least three plasma proteins, i.e. the Hageman factor, prekallikrein and high molecular weight kininogen (for review see Murano 1978). The role of the factor XII mediated fibrinolysis in the pathophysiology of thromboembolic diseases is not properly understood. Thromboembolic diseases in patients with Hageman trait and in patients with acquired inhibitors against the activation of factor XII have been described (Ratnoff et al 1968, Hedner & Nilsson 1976, Mueh et al 1980, Dyerberg & Stoffersen 1980).

7

These various mechanisms of plasminogen activation results in the formation of plasmin, which digests the insoluble fibrin into soluble fragments known as fibrin/fibrinogen degradation products (FDP) (Marder & Shulman 1969, for reviews, see Gaffney 1979 and Castellino 1981).

### Plasminogen and plasmin

The liver appears to be the major site of biosynthesis of human plasminogen (Robbins 1981). The concentration in plasma is 10-20 microgram/100 ml. A raised level of plasminogen is found in conditions associated with increased amounts of acute-phase-reactants, which is seen after trauma, infection and surgery (Lackner & Javid 1973).

Plasminogen is a single chain  $\beta_2$ -glycoprotein with a molecular weight of about 90,000, containing 791 amino acid residuals (Robbins & Summariia 1963, Wiman 1978, Sottrup-Jensen et al 1978). The amino acid sequence of the molecule is determined mainly by Magnusson and co-workers (Sottrup-Jensen et al 1978) and by Wiman (1978). The molecule consists of three parts, the N-terminal part, the intermediate part and the C-terminal part. The intermediate part contains five homologous triple loop structures, also named "kringles". The plasminogen molecule is stabilized by 24 disulphide bridges.

It is the intermediate part of the plasminogen molecule which binds to fibrin and the inhibitor  $\alpha_2$ -antiplasmin ( $\alpha_2$ AP). This part of the molecule has also so-called lysine-binding sites, which have affinity for  $\omega$ -aminocarboxylic acids, i.e. lysine epsilon-amino-caproic acid and tranexamic acid (reviewed by Wiman et al 1979). This part of the molecule is the main structure for plasminogen activation.

Native plasminogen has a glutamic acid at the N-terminal part of the molecule (Wallén & Wiman 1970) and is therefore called glu-plasminogen, which has a low affinity for fibrin (Thorsen 1975, Rakoczi et al 1978). By proteolytic degradation of the glu-plas-

minogen with removal of peptide material, native plasminogen is converted to another plasminogen with a lysine binding site of the molecule, lys-plasminogen. In contrast to the native glu-plasminogen lys-plasminogen has a high affinity for fibrin (Wiman & Wallén 1975, Thorsen 1975, Rakoczi et al 1978). The activation of lys-plasminogen to plasmin by urokinase is about 20 times faster than the activation of glu-plasminogen (Thorsen et al 1974).

Plasmin is formed from plasminogen by the action of activators splitting off at least two peptide bonds of the plasminogen molecule (Robbins et al 1967, reviewed by Wiman 1978). The C-terminal part of the molecule corresponds to the proteolytic part of plasmin. A two-chained plasmin molecule is formed when plasminogen is activated. The chains, which are called heavy and light chains, respectively, are linked together by two disulphide bonds (Wiman 1977). The most active site of the plasmin molecule is the light chain (Robbins et al 1967, Groskopf et al 1969, Wiman 1977, Sottrup-Jensen et al 1978). The inactivation of plasmin by  $\alpha_2$ AP occurs at the light chain of the plasmin molecule (Wiman & Collen 1977).

Plasmin is a non-specific proteolytic enzyme with a broad specificity, but the main target in vivo is fibrin. However, plasmin also influence other coagulation factors i.e. fibrinogen, factor V, factor VIII and prothrombin (Donaldson 1960, Pasquini & Hershgold 1973) as well as the activation of several complement zymogens (reviewed by Castellino 1981).

#### Plasminogen activation

Several hypotheses have been put forward to explain how plasmin uses fibrin as a substrate. Müllertz (1956) and Astrup (1956b) showed that activator is present in low concentrations bound to inhibitor in the circulating blood, but is adsorbed to and accumulated on the fibrin surface. The effect of the inhibitors against activation and against plasmin is reduced by the adsorption of activator and plasmin to fibrin.



Alternative possibilities for the plasminogen activation have been put forward by Alkjaersig et al (1959), Ambrus & Markus (1960) and Chesterman et al (1972). With histochemical technique Todd & Nunn (1967) showed that plasminogen activators are adsorbed from the endothelium of the vessel wall and incorporated in the thrombus.

Tissue plasminogen activator obtained from pig heart has strong affinity for fibrin (Thorsen et al 1972) and human tissue plasminogen activator (PA) behaves in a similar way (Thorsen 1977, Wallén et al 1981, Rijken & Collen 1981). Fibrin markedly enhances the activation of plasminogen by tissue activator and human vascular activator (Wiman & Wallén 1977, Thorsen & Müllertz 1979). This seems to be due to the adsorption of both plasminogen and activator to the fibrin net work whereas urokinase and streptokinase do not have such specific affinity for fibrin (Chesterman et al 1972, Thorsen et al 1972, Wijngaard 1979, Rijken & Collen 1981).

According to recent data, a hypothesis for the activation of the fibrinolytic system is as follows. In the absence of fibrin all formed plasmin is neutralized by  $\alpha_2$ AP (Thorsen & Müllertz 1979) but, when a thrombotic formation is present, which contains fibrin, both plasminogen and activators are adsorbed to the fibrin surface (Thorsen et al 1972, Thorsen 1977, Wiman & Collen 1978). By small amounts of activators glu-plasminogen is converted to lys-plasminogen which is firmly bound to the fibrin molecule, and make the complex inaccessible to  $\alpha_2$ AP (Thorsen 1975, Wiman & Collen 1978). As pointed out the plasminogen activators also adsorb to fibrin and convert lys-plasminogen to plasmin, which digests fibrin. The insoluble fibrin is digested to soluble fragments (FDP). This formation is followed by release of activators, plasminogen and plasmin, and the plasmin is rapidly and irreversibly neutralized by  $\alpha_2$ AP. When  $\alpha_2$ AP is consumed another inhibitor,  $\alpha_2$ -macroglobulin, can neutralize further plasmin (Wiman & Collen 1978, Thorsen & Müllertz 1979). This mode of action is an extrinsic clot lysis of fibrin, which contrasts with the earlier hypothesis by Alkjaersig et al (1959). The extrinsic clot lysis theory has been broadly accepted but it should be pointed out that the exact mechanism of the activation of plasminogen in vivo remains unsettled.

## Inhibitors

Inhibitors of the fibrinolytic system consist of two groups, one inhibiting the plasminogen activation and the other acting as antiplasmin.

In the circulation there are inhibitors of plasminogen activators (Müllertz 1956, Hedner et al 1970), which inhibit urokinase-induced fibrinolysis. Hedner & Martinsson (1978) showed that the plasminogen activator inhibitor does not inactivate urokinase in a purified system but inhibits factor XIIa and furthermore is immunochemically different from  $\alpha_2$ AP (Hedner & Collen 1976). In spite of extensive research inhibitors of plasminogen activators are, however, less well defined, which is partly due to the fact that these inhibitors are very sensitive to the presence of plasmin inhibitors and are therefore difficult to assess. C<sub>1</sub>-inactivator is another inhibitor of plasminogen activators (Kluft 1977).

The second group of inhibitors are the antiplasmins (Ratnoff et al 1954). Norman & Hill (1958) isolated two fractions which inhibited plasmin. One fraction was a fast reacting inhibitor, which migrated like an  $\alpha_2$ -globulin, and the second fraction an  $\alpha_1$ -globulin which inhibited plasmin slowly. These enzymes were named  $\alpha_2$ -macroglobulin and  $\alpha_1$ -antitrypsin, respectively. Isolation and characterization of these two inhibitors were described by Shultze et al (1963) (reviewed by Laurell & Jeppsson 1975).  $\alpha_2$ -macroglobulin inactivates plasmin formed in the excess of the inhibitor capacity of  $\alpha_2$ AP (Collen 1976, Müllertz & Clemmensen 1976).  $\alpha_2$ -macroglobulin inhibits not only plasmin, but also thrombin, trypsin, chymotrypsin, elastase and kallikrein (reviewed by Ganrot 1967, Steinbuch et al 1972).

In 1976 four groups of researchers could independently isolate a stable fast-reacting plasmin inhibitor in human blood which has been called  $\alpha_2$ -antiplasmin ( $\alpha_2$ AP) (Collen 1976, Moroi & Aoki 1976, Müllertz & Clemmensen 1976, Saldeen 1976). This inhibitor is considered the major physiological inactivator of plasmin. Its molecular weight is about 70,000 (Wiman & Collen 1977) and it consists of a single chain glycoprotein.

The binding of  $\alpha_2$ AP to plasmin is dependent of the availability of free lysine-residues in the plasmin molecule. In the presence of such residues, plasmin is rapidly inactivated by the inhibitor. Conversely when the lysine residues are blocked by fibrin, inactivation by  $\alpha_2$ AP is reduced. The reaction between plasmin and  $\alpha_2$ AP occurs in two steps, a very fast reversible complex followed by a slower irreversible formation (Wiman & Collen 1978). Clemmensen (1979) produced evidence of the presence of two different molecular forms of  $\alpha_2$ AP in plasma, both inactivating plasmin.

Other antiplasmins are inter- $\alpha_1$ -trypsin inhibitor (Steinbuch & Loeb 1961) and antithrombin III (Abildgaard 1967, Highsmith & Rosenberg 1974). The latter is the main physiological inhibitor of thrombin and factor X activator in the coagulation system (Abildgaard 1979).

Human tissue cell cultures yield inhibitors of plasminogen activators (Bernik & Kwaan 1971). In smooth muscle cells in the vessel wall Brakman et al (1966) found an inhibitor of tissue-induced fibrinolysis. Loskutoff & Edgington (1977) and Levin & Loskutoff (1979) demonstrated cellular activators and inhibitors of fibrinolysis in cell cultures of the endothelium of the vessel wall. The relation between activators and inhibitors may change with the stage of growth of the cells.

Using a fibrin slide "sandwich" technique, Noordhoek-Hegt & Brakman (1974) demonstrated the existence of inhibitors of the fibrinolytic system in the vessel wall. With the histochemical method they used the inhibitors were found to be localized to the intima and media of the vessel wall. An inverse relationship exists between inhibitors and activators of the fibrinolytic system (Noordhoek-Hegt 1977).

The physiological balance between activators and inhibitors of the fibrinolytic system can be altered in many pathologic conditions (Aoki 1979). Inhibitors of the fibrinolytic system are increased after surgery (Tsitouris et al 1961, Ygge 1970, Gallus et al 1973, Griffiths et al 1977b, Griffiths 1979). In a few studies inhibitors have been found to be of etiological importance to the development of venous thrombosis (Nilsson et al 1961, Brakman et

al 1966, Nilsson 1967, Pandolfi et al 1970, Alexandre et al 1980). On the other hand a low content of  $\alpha_2$ AP which is extremely rare causes severe bleeding (Koie et al 1978, Sakata & Aoki 1982).

There are synthetic inhibitors of the fibrinolytic system, i.e. epsilon-amino-caproic-acid (EACA) (Okamoto et al 1959, Nilsson et al 1960), tranexamic acid (AMCA) (Okamoto & Okamoto 1962, Anderson et al 1965) and p-amino-methyl-benzoic acid (PAMBA) (Markwardt et al 1967). The main action of the aminocaproic acid compounds is to compete with lysine binding sites on the plasminogen and the plasmin molecule (reviewed by Castellino 1981). They inhibit the activation of plasminogen by streptokinase, urokinase and tissue activator, and cause a stoichiometric inhibition of plasmin with the formation of an inactive complex between plasmin and aminocaproic acid (Thorsen 1978). The synthetic inhibitors are of importance in clinical conditions in which the fibrinolytic system is enhanced (reviewed by Nilsson 1979a).

## Activators

### Tissue activators

Human uterus, adrenal glands, lymph nodes, prostate, thyroid, lungs and ovaries have a high concentration of activators, while little or no activity is found in the testis, spleen and liver (Albrechtsen 1957). The vascular endothelium is the main source of tissue activators (Todd 1959, 1961, Kwaan & Astrup 1963, Warren 1964, Pandolfi 1967, Rijken et al 1979).

The activators are confined to the endothelial cells and in large vessels also to the vasa vasorum in the adventitia (Todd 1959, Kwaan & Astrup 1964, Kwaan 1966, Pandolfi et al 1967, Yao et al 1974). With a histochemical fibrin slide technique Todd (1958, 1959) localized the activators in tissues. With this method a thin layer of fibrin is produced by adding fibrinogen to a film of thrombin on a glass slide. Sections of tissue are collected to the surface of the film and afterwards incubated, which reveal zones of digestion of the fibrin where activators are present.

With this technique activators are localized to the vascular endothelium, an observation which later has been confirmed by several others who used the same technique (Warren 1964, Kwaan 1966, Pandolfi et al 1967).

The use of histochemical and extraction techniques obtained from necropsy specimens has resulted in conflicting data concerning the plasminogen activator (PA) activity in the intima of the vessel wall. Thus, several workers have found little or no activity in the intima of arteries but a high activity in large veins (Lieberman & Kellogg 1961, Coccheri & Astrup 1961, Astrup 1966, Noordhoek-Hegt 1976). Others, however, have found an activity also in the intima of arteries (Constantini et al 1972, Onoyama & Tanaka 1969, Donner et al 1977).

By preparing sheets of endothelial cells with a microtome ("häutchen" preparations) Todd (1961) and Warren (1964) demonstrated activators localized to the intima of the vessel wall. By modification of Todd's histochemical method it has been possible to semiquantify the PA activity in the vessel wall (Kwaan & Astrup 1963, 1967b, Pandolfi et al 1967, Peterson 1968, Constantini et al 1969b, Risberg 1976, Browse et al 1977a, Smokovitis 1979).

Activators may also derive from mesothelial cells, cells lining synovial membranes and in epithelial cells and occur in erythrocytes and leukocytes (reviewed by Astrup & Thorsen 1972, Ogston 1978 and Whitaker et al 1982). Assay of subcellular fractions has revealed activators in lysosomal and microsomal fractions (Lack & Ali 1964, Nakahara & Celander 1968). In a study using ultrastructural morphology Warren & Khan (1974) found that activators were localized to the cytoplasm in endothelial cells, a finding which later was verified by immunological methods (Todd & Hargreaves 1975, 1978).

As mentioned above cellular activators of the fibrinolytic system have also been demonstrated in cell cultures of the vessel wall, where synthesis and storage of PA has been localized (Bernik & Kwaan 1967, 1969, Pandolfi 1970, Åstedt & Pandolfi 1972, Loskutoff & Edgington 1977, Loskutoff 1979, Rijken et al 1980).



Using an extraction technique with high molar potassium thiocyanate, it was possible to assay the tissue activator in animal and human specimens by the fibrin plate method described by Astrup & Müllertz (1952) (Astrup & Albrechtsen 1957, Albrechtsen 1959, Astrup et al 1959).

Further development within this field includes the measurement of the PA content by estimating the radioactivity released from  $^{125}\text{I}$ -tagged fibrin clots (Johnson & Mansfield 1978) and the use of chromogenic substrate (Risberg & Claeson 1979).

With immunochemical assays of fibrinogen/fibrin degradation products it is also possible to quantify fibrinolytic activators released from tissue cultures (Åstedt & Pandolfi 1972, Pandolfi et al 1974). Since the PA can now be purified it will be possible to assay the activity with immunoradiometric assay but still no method is applicable to determine tissue PA.

Using an extraction technique and gel filtration Kok & Astrup (1969) isolated PA from ovaries from pregnant pigs and estimated the molecular weight to be about 60,000. This activator was different from that of urokinase, which have been found to have a molecular weight of 54,000 (White et al 1966). Furthermore, Kok (1979) distinguished between three types of plasminogen activators in extracts from human uterine tissue, i.e. a stable activator, a labile activator and an activator similar to urokinase. In further studies it has been demonstrated that the vascular activator is different from human urokinase in terms of immunologic reaction, enzymatic activity and molecular weight (Aoki & von Kaulla 1971a, Camiolo et al 1971, Thorsen et al 1972, Wiman & Collen 1978, Wijngaard 1979, Rijken et al 1979, 1981, Rijken & Collen 1981). The tissue activator is immunologically related to the vascular activator (=plasminogen activator) released from the vascular wall during perfusion of blood vessels by the technique described by Aoki and von Kaulla (1971b) and during venous occlusion (Rijken et al 1979, Holmberg et al 1982b).

Since it is now known that tissue activators adsorb strongly to fibrin, it has been possible to purify and to characterize PA. Using this property Wallén et al (1981) succeeded in purifying

porcine plasminogen activator and human uterine tissue activator based on an immunosorbent technique. These two activators may occur in two different forms, i.e. as a single polypeptide chain (m.w. 64,000) which easily can be converted to a two-chain variant (m.w. 32,000) with different enzymatic properties (Wallén et al 1981, Rijken & Collen 1981). The original tissue activator is formed as an one-chain component, which may be transformed into a two-chain variant by the action of plasmin or trypsin (Rijken et al 1982).

Recently Rijken & Collen (1981) purified and characterized plasminogen activator from human melanoma cell cultures and compared this activator with urokinase and with purified uterine tissue activator. The melanoma activator shows the same electrophoretic mobility and the same immunologic properties as the uterine tissue activator, suggesting that the two activators have the same molecular weight. These two activators also have comparable amino acid sequences.

### Urokinase

Urokinase is a trypsin-like protease which probably originates from the urinary outflow pathways (Bernik & Kwaan 1969, Shakespeare & Wolf 1979). It occurs in different molecular forms with different molecular weights. Purification by chromatography on benzamidine-Sepharose has revealed two main components with molecular weights of 54,000 and 31,000, respectively (Holmberg et al 1976). It can be obtained in urine, from cultured kidney cells and be produced by various tumor tissues (Åstedt & Holmberg 1976). Urokinase-like activation has been found in normal human endometrium as well as in malignant endometrium (Holmberg & Åstedt 1981).

As mentioned earlier urokinase differs from the vascular PA in antigenic characteristics (Wiman & Wallén 1977, Rijken et al 1979) and its enzyme specificity, particularly with respect to the activation of fibrin associated plasminogen (Camiolo et al

1971). Urokinase directly converts plasminogen to plasmin. The systemic plasmin so formed is rapidly neutralized by  $\alpha_2$ AP, but urokinase administered in excess results in formation of free plasmin, which can attack thrombi formations and circulating fibrinogen. Urokinase is a powerful thrombolytic agent in arterial as well as in venous thrombosis (Tsapogas 1964, Kakkar & Scully 1978, Schmitz-Huebner & van de Loo 1981).

### Streptokinase

In 1933, Tillett & Garner showed that an extracellular protein produced by hemolytic streptococci was capable of causing lysis in clots containing human fibrinogen. Later, Müllertz & Lassen (1953) showed that human, but not bovine plasminogen, was activated by streptokinase. According to these authors it reacts with a component in the euglobulin fraction (proactivator) to form an activator capable of converting plasminogen to plasmin. Streptokinase (SK) forms an equimolar complex with plasminogen (SK-plasminogen) or plasmin (SK-plasmin), which converts plasminogen to plasmin. Like urokinase, streptokinase is effective when all  $\alpha_2$ AP is neutralized (for review, see Castellino 1979, 1981).

### Blood activators and PA release

The existence of blood activators was first suggested by Mole (1948). As pointed out the activators are most likely derived from tissue activators in the vessel wall, but may also originate from precursors in the blood after contact activation of factor XII mediated fibrinolysis and from urokinase (Murano 1978, Shakespeare & Wolf 1979). It is difficult to assess labile blood activators in normals because they occur only in trace amounts in the circulation (Andersson et al 1962, Robertson et al 1972b). Aoki (1974) found identity between activators from postmortem perfusates of the vascular tree and blood activators.

There is a diurnal fluctuation in the circulating activity (Rosing et al 1970, Kluft 1978) which can be markedly raised by

various stimuli, such as exercise, anxiety, surgical operations and anoxia (for review, see Nilsson 1975).

Venous occlusion of a limb is followed by a marked local increase in the fibrinolytic activity (Nilsson & Robertson 1968, reviewed by Nilsson 1975) and furthermore, Shaper et al (1975) demonstrated a correlation between the resting level of circulating fibrinolytic activity and the response elicited by venous occlusion. Measurement of circulating fibrinolytic activity after standardized venous occlusion of the upper limbs has been found to be a simple and indirect assessment of the fibrinolytic capacity (Robertson et al 1972b).

There is convincing evidence that the increase of the fibrinolytic activity in the blood after venous stasis and physical exercise is achieved by release of activators from the endothelium (Rijken et al 1980, Nilsson et al 1982).

With an immunoradiometric assay (IRMA) Holmberg et al (1982b) investigated PA in plasma before and after venous occlusion, administration of desmopressin (DDAVP) and physical exercise. Urokinase-like material was determined with a conventional radioimmunoassay. A pronounced increase of tissue activator was found after venous occlusion, after infusion of DDAVP and after physical exercise but no urokinase was detected. This study provides additional evidence that the fibrinolytic activity in blood results from PA released from the vascular wall.

Administration of vasoactive drugs, such as adrenalin, nicotinic acid, vasopressin (for review, see Nilsson 1975 and Radcliffe & Heinze 1978) and its synthetic analogue DDAVP enhance the fibrinolytic activity in blood (Mannucci et al 1975, Nilsson et al 1982, Holmberg et al 1982a). Furthermore, adrenalin, nicotinic acid and DDAVP stimulate the release of factor VIII related antigen (VIIIIR:Ag) (Mannucci et al 1975, Åberg et al 1979, Nilsson 1979b, Nilsson et al 1980b, 1982), which is also synthesized in the vessel endothelium (Bloom et al 1973, Hoyer et al 1973, Holmberg et al 1974). Recently Nilsson and co-workers (1982) found a correlation between the release of PA and VIIIIR:Ag after administration of DDAVP and after venous occlusion.

The release mechanism of PA from the endothelial cells is only partly known. With histochemical technique Constantini et al (1969a) demonstrated that venous occlusion is attended by a transfer of PA from the adventitia to the intima in veins from the upper limb. Browse et al (1977a) demonstrated a correlation between spontaneous and poststasis blood fibrinolytic activity on one hand and vascular fibrinolysis on the other. In two experimental studies in rats, Szczepanski & Nilsson (1970) and Risberg et al (1981) found a decreased PA activity in veins after venous occlusion. Both used histochemical methods. Also in pigs, Wu & Mansfield (1979) found that repeated venous stasis caused a marked decrease in the fibrinolytic activity of the veins demonstrable histochemically.

Many alternative explanations have been put forwards as for the release mechanism of PA from the vessel wall. Thus, a catecholamine induced release has been postulated by Cash (for review, see Cash 1978). There is also evidence that norepinephrine is involved in the release of antidiuretic hormone (Barker et al 1971) and this hormone also causes an enhancement of fibrinolytic activity. On the other hand it has been demonstrated that the fibrinolytic activity induced by various stimuli can be blocked only partially by  $\beta$ -receptor blocking drugs or is not prevented at all (Ponari et al 1973). Gader et al (1974) suggested that the PA release is related to different receptor sites.

There is no strict correlation between the release of the catecholamines and the activation of fibrinolysis following physical exercise (Hawkey et al 1975) or electroshock (Pina-Cabral & Rodrigues 1974), which suggests additional control mechanisms of the PA release. However, under physiological conditions the mediator may partly consist of catecholamines (Cash 1975). But other neurohumoral mechanisms are also thought to be of importance (Schneck & von Kaulla 1961, Cash 1978). The above mentioned stimuli have one effect in common, they all produce a change in the caliber of the blood vessels. Such changes in the vasoactive motility in the vessel wall is followed by the release of fibrinolytic activity (Holemans 1965a,b, Nilsson & Pandolfi 1970). There are, however, powerful vasoactive drugs that do not demonstrably stimulate any release of the plasminogen activators, i.e.



histamine and serotonin (Thomson et al 1964). Moreover, DDAVP, which lacks vasopressor properties has been shown to produce a marked fibrinolytic response (Mannucci et al 1975, Nilsson et al 1982, Holmberg et al 1982b).

Another trigger mechanism to the activation is mechanical injury (Chakrabarti et al 1963, Acinapura et al 1966). With histochemical technique Todd (1961) showed that distorted and dislocated endothelial cells had more fibrinolytic activity than their apparently intact neighbours. Silver (1969) demonstrated that direct vascular trauma is associated with reduced fibrinolytic activity in the vessel wall. In an experimental study in rats Risberg & Bylock (1981) found that endothelial injury is followed by a decrease of PA in the vessel wall, which is seen with histochemical technique.

#### BACKGROUND TO THE PRESENT INVESTIGATION

Several studies have indicated the significance of a defective fibrinolytic system in the development of venous thrombosis (Pandolfi et al 1969, Isacson & Nilsson 1972, Nilsson & Isacson 1973, Nilsson et al 1975, Browse et al 1977a, Nylander et al 1977, Ciavarella et al 1979, Nilsson et al 1981, Sundqvist et al 1981, Ljungnér et al 1982). Thrombi are usually localized to the legs where a low PA activity has been found in superficial leg veins (Pandolfi et al 1969). Nothing is known, however, about the PA activity in deep veins where thrombi of clinical importance are localized. It is therefore of importance to investigate if any correlation exists in the PA activity between superficial and deep leg veins, since it is not possible to obtain specimens from deep vein by routine.

The fibrinolytic capacity estimated after venous occlusion is three to four times higher in the arms of healthy volunteers than it is in the legs (Nilsson & Robertson 1968), and there is also a correlation in the local plasma fibrinolytic activity between the arms and the legs (Robertson et al 1972a). In superficial hand

veins the PA activity is higher than it is in superficial foot veins in healthy subjects (Pandolfi et al 1968, 1969). It is not known, however, whether or not any correlation exists in the PA activity between superficial hand and foot veins in healthy subjects and in patients with leg vein thrombosis. This question has a clinical implication since biopsy specimens are obtained by routine from superficial hand veins.

The physiological role of the arterial fibrinolytic activity is not known and the significance of the fibrinolytic system in the pathogenesis of atherosclerosis is still the subject of wide debate (Astrup 1956a, Peabody et al 1974, Kwaan 1979, Smokovitis 1980). In contrast to the overwhelming number of studies dealing with the PA in veins there is a definite need of systematic studies of arterial PA, as opinions of this activity vary widely mainly owing to the fact that most studies have been carried out on postmortem arteries. Except for data from one small series in diabetics, where a correlation in the PA activity between temporal arteries and superficial hand veins was observed (Almér et al 1975a), nothing is known about the correlation, if any, in the PA activity between arteries and veins. Nor is it known whether there is any correlation in the activity between arteries obtained from different anatomical regions in a given patient.

The pathophysiological role of the fibrinolytic system in the etiology of postoperative venous thrombosis has received little attention. It is known that major surgery is accompanied by alterations in plasma fibrinolysis (for review, see Risberg 1978, 1979) and also a postoperative decrease in the PA content of superficial hand veins (Åberg & Nilsson 1978, Isacson & Ljungnér 1978). It is not known, however, when such a decrease appears, how long it lasts, or which role risk factors considered to predispose to postoperative DVT play in the postoperative PA response. As transvesical prostatic surgery (TVP) and trans-urethral resection of the prostata (TURP) are relatively well standardized operations on one and the same organ with highly different risk for DVT (reviewed by Rose 1979), it was also thought worth while to compare the PA response after these two types of surgery.

Intermittent pneumatic compression (IPC) is one of several mechanical methods which has proved effective in the reduction of postoperative venous thrombosis (for review, see Bergqvist 1979). In a few studies graduated static compression has significantly reduced the frequency of postoperative DVT (Holford 1976, Scurr et al 1977, Bolton 1978).

The mechanism by which these methods exert the prophylactic effect is not fully understood. Different systems of IPC with different effect on the venous flow have the same prophylactic effects (Ah-See et al 1976). As it is known that venous occlusion increases the fibrinolytic activity, it has been suggested that IPC may stimulate the fibrinolytic system. Some studies have dealt with this problem, but the results obtained so far are conflicting (Allenby et al 1973, Knight & Dawson 1976, Browse 1976, O'Brien et al 1979, Tarnay 1980).

## PURPOSE

The purposes of this investigation were

1. To assess the PA activity and to analyse the correlation in the PA activity between
  - a) superficial, muscle and deep leg veins in patients undergoing a leg amputation (I),
  - b) superficial hand and foot veins in patients with and without previous venous thrombosis (II) and
  - c) different arteries and concomitant veins in patients with various diseases according to purpose 2 and in healthy subjects all undergoing surgery (VI)
2. To determine the PA activity in arteries and veins in patients with overweight, diabetes mellitus, uraemia and arteriosclerosis (I, VI)
3. To study the postoperative PA response in superficial hand veins in patients undergoing various surgical operations and to determine when and for how long this response is influenced by the surgical trauma (III, IV)
4. To assess the postoperative PA response in patients developing venous thrombosis and to determine the PA response in relation to various risk factors considered to predispose to postoperative DVT (III, IV)
5. To evaluate the effect of intermittent pneumatic compression (IPC) and graduated static compression on the fibrinolytic activity and factor VIII in the local and general circulation and also the PA activity in superficial vein walls (V)

## MATERIAL AND METHODS

### Patient material

More detailed information is given in the individual publications (I - VI)

Table I

| Publication | Total number<br>of patients |                                                                                                                                                                                                                          |
|-------------|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| I           | 35                          | Above knee amputation, 9 patients.<br>Below knee amputation, 26 patients.<br>Arterial insufficiency existed in all<br>patients except one. Diabetes mellitus,<br>16 patients                                             |
| II          | 68                          | 46 patients had previous DVT; at<br>least three months before the pre-<br>sent investigation. All thrombi were<br>localized to the legs                                                                                  |
| III         | 108                         | All patients underwent major surgery<br>and were given either low dose hepa-<br>rin or dextran 70                                                                                                                        |
| IV          | 56                          | 36 patients underwent TVP (13 general<br>anaesthesia, 23 epidural analgesia).<br>20 patients underwent TURP (10 epi-<br>dural and 10 spinal analgesia). All<br>patients received low dose heparin<br>and tranexamic acid |
| V           | 50                          | 23 patients received slow and 15<br>quick IPC, 12 patients had graduated<br>compression stockings                                                                                                                        |
| VI          | 115                         | Arteries and concomitant veins were<br>obtained from popliteal, pudendal,<br>inferior epigastric, iliac and renal<br>vessels, 45 patients had diabetes<br>mellitus, 23 arteriosclerosis and 52<br>terminal uraemia       |



## Definitions

Obesity. At least 10 % overweight according to the criteria of Bengtsson et al (1981).

Diabetes mellitus. Chronic hyperglucemi which make treatment with insulin or oral drugs necessary.

Uraemia. Chronic renal diseases in patients undergoing a kidney transplantation.

Arteriosclerosis. Atherosclerotic leg arteries in patients undergoing a leg amputation.

Major surgery. An operation lasting more than 60 min.

Minor surgery. An operation lasting 60 min or less.

## Laboratory methods

### Method of PA determination

The PA activity of biopsy specimens was assessed by a histochemical method described by Todd (1958, 1959) and later modified by Pandolfi et al (1967, 1968). Segments, 0.5 - 1.0 cm long were obtained with great care and with a minimum of trauma under local anaesthesia (Lidocain-Chloride 0.5 %) (I - IV), under general or spinal anaesthesia (I - VI). The specimens were hermetically enclosed in "parafilm" and frozen to -70°C and stored at this temperature until the preparations were performed.

Sections were cut into 0.8 µm on cryostate microtome (Harris' cryostate) using a freezing gel in cryoform. The sections were collected on glass slides which were previously washed in alcohol and incubated in close contact with a thin fibrin film rich in plasminogen. Bovine fibrinogen was prepared according to Brakman's (1967) modification of Astrup & Müllertz (1952) double ammonium sulphate precipitation method. The same fibrinogen batch was used throughout the study. The film was obtained by mixing bovine fibrinogen (1 %) in phosphate buffer (pH 7.8, ionic strength 0.15)

which was coagulated with thrombin (Topostasine, Roche, 20 NIH U/ml). By means of a glass rod the mixture (60  $\mu$ l of fibrinogen solution and 10  $\mu$ l of thrombin) was spread over an area of 10 cm<sup>2</sup> of the slide, resulting in a fibrin film about 70  $\mu$ m thick. Four slides were prepared for each specimen. The slides were placed horizontally for 30 min to consolidate the fibrin film. This was done in a moist chamber at a temperature of +19°C or lower. The slides were afterwards incubated in another moist chamber at +37°C for 10, 20 and 30 min, respectively. After the incubation periods, the slides were fixed in formalin (10 %) for 10 min, followed by staining with Harris' hematoxylin and in Giemsa solution (20 %) for at least 20 min for each solution. Finally the slides were mounted in glycerol-gelatin. Fibrinolysis appeared as white areas in the fibrin film at the active sites of the sections.

Three fairly distinct degrees of fibrin digestion were recognized and quantified as

grade I: Small punctate area of lysis in the majority of the sections,

grade II: Larger, sometimes confluent areas of lysis,

grade III: Massive fibrin digestion of all fibrin in contact with the sections

A slide without lysis was graded 0, a grade I slide was allotted 1 point, a grade II slide 2 points, and a grade III slide 3 points. Degrees of digestion between 0-I, I-II, and II-III were allotted 0.5, 1.5 and 2.5 points, respectively. The sum of the points from four slides was expressed in arbitrary units (maximally 12 arbitrary units) and taken as a measure of the PA activity of the specimen. In our laboratory the median value of superficial hand veins of 60 healthy volunteers aged 17-70 years is 7.5 arbitrary units (range 6-10 units) (Nilsson & Pandolfi 1976). No normal value is available from arterial specimens from living humans.

## Reproducibility of the method

Table II shows the reproducibility of the PA assessment in vein walls according to Pandolfi's modification of Todd's fibrin slide technique. The figures in the table show on one hand the variation in the PA assessment performed by the same observer at two occasions by an interval of three months between the examinations (A), as well as the variation in the PA activity assessed independently by different observers (B, C).

Table II

| Examination              | Total number of slides | Percentage of fibrin slides with variation of PA activity in arbitrary units by: |     |      |
|--------------------------|------------------------|----------------------------------------------------------------------------------|-----|------|
|                          |                        | 0.5                                                                              | 1.0 | 1.5  |
| A. Pandolfi et al (1972) | 496                    | 11.8                                                                             | 3.2 | 0.06 |
| B. Coag. lab. (1981)     | 116                    | 13.6                                                                             | -   | -    |
| C. Present investigation | 1840                   | 14.4                                                                             | 4.4 | -    |

In the present investigation altogether 4372 fibrin slides were examined by myself and in paper II and III 1840 fibrin slides were examined by two observers according to Table II.

### Influence of local analgesia

To find out whether local analgesia influenced the PA activity, five patients undergoing varicose vein surgery were examined. Two biopsy specimens were obtained from each patient from the same foot before the operation. One specimen was obtained under general anaesthesia and a second specimen after infiltration with 0.5 % Lidocain Chloride. No difference in the PA activity was found in specimens obtained by the two techniques (Table III).

Table III

The PA activity in arbitrary units in foot veins obtained under different techniques of anaesthesia in 5 patients

| Patient number | PA activity (arbitrary units) in foot veins |                                         |
|----------------|---------------------------------------------|-----------------------------------------|
|                | General anaesthesia                         | General anaesthesia and local analgesia |
| 1              | 3.5                                         | 3.0                                     |
| 2              | 4.0                                         | 4.0                                     |
| 3              | 6.0                                         | 5.5                                     |
| 4              | 6.0                                         | 6.5                                     |
| 5              | 7.0                                         | 7.0                                     |

### Blood samples

Blood (4.5 ml) was collected without venous occlusion in vacutainers which were treated by silicon and contained 0.5 ml 3.8 % citrate solution. Citrated plasma was prepared according to Nilsson et al (1957). Sampling was always done from an antecubital vein before and immediately after finished IPC and treatment by stockings, respectively. In patients where IPC was used to the arm, samples were obtained from the compressed side. In four patients a catheter was placed in the femoral vein and blood samples were obtained during the different compression phases.

Factor VIII activity (VIII:C) was determined as described by Nilsson et al (1957). Normal range: 60-160 %.

Factor VIII related antigen (VIIIIR:Ag) was determined with Laurell's electroimmuno assay technique (Holmberg & Nilsson 1973). Normal range: 50-160 %.

Fibrinolytic activity was measured on unheated human fibrin plates with citrated plasma and resuspended euglobulin precipitate as test solutions (Nilsson & Olow 1962). Normal range, citrated plasma: 0-59 mm<sup>2</sup> and resuspended euglobulin precipitate: 44-152 mm<sup>2</sup>.

#### Fibrinogen uptake test

The <sup>125</sup>I-labelled fibrinogen uptake test was used as a screening method for the diagnosis of postoperative deep vein thrombosis in 31 patients. The first measurement with a scintillation counter was made preoperatively, and thereafter postoperative measurements were made every day for one week or until discharge if earlier. Radioactivity was measured at 9 points on each leg, and was correlated with heart activity. An increase of 20 % or more compared to adjacent points on two consecutive measurements was diagnosed as a thrombosis. All patients with positive tests were investigated with an ascending phlebography. The technique has been described by Negus et al (1968).

#### Mechanical thromboprophylactic methods

The pumps used were Flowtrone-Aire AC/200 and Flowpulse 1100 (Huntleigh Medical Limited, England). The former pump produced slow IPC with an inflation period of about two minutes followed by a deflation period of about the same duration, after which a new cycle was started. Quick IPC was performed by the latter pump, with a three seconds' inflation period followed by a 20 seconds' deflation period. The pressure used in both pumps was 40 mm Hg.

Two types of static compression stockings were used with ankle pressures of 18 and 35 mm Hg, respectively.

### Statistical methods

Statistical calculations were performed by Mann-Whitney's rank sum test, Wilcoxon's rank sum test of paired observations, Student's t-test of paired observations (when  $n > 26$ ), Chi-Square test (Yate's correction  $n < 5$ ), Fisher's exact test and the sign test.

Correlation coefficients were calculated according to Spearman's rho ( $\rho$ ) (Siegel 1956). The differences between results were considered to be statistically significant when  $p < 0.05$ .

## RESULTS

### PA activity and correlation analysis between the activity in different vessels

The PA activity in the great saphenous vein was significantly lower than the activity in deep and muscle veins obtained from the same level (I). There was no difference in the activity between deep and muscle veins. The PA activity in the great saphenous vein was higher in 9 patients where the specimens were obtained above the knee than the activity in the same vein obtained below the knee in 26 patients (I). No such difference was found in the activity between deep veins obtained above and below the knee.

The PA activity in superficial hand veins was the same as the activity in superficial foot veins in 39 (57 %) patients. A difference in the activity by more than 0.5 arbitrary unit between hand and foot veins was found in 29 patients, 26 (38 %) of whom had a higher activity in the hand veins (II). Patients with venous thrombosis at least three months before the investigation had a lower activity in superficial foot veins compared with the acti-



vity in foot veins in patients without thrombosis (II).

The PA activity in arteries and concomitant veins did not differ in various anatomical regions, except in the inferior epigastric vessels, where the activity was significantly higher in the veins (VI). Age and sex did not influence the activity in the various arteries examined.

As seen from Table IV, there was a correlation in the PA activity between superficial and deep leg veins, between deep and muscle leg veins, between superficial hand and foot veins and between various arteries.

Table IV

Total number of biopsy specimens (n), correlation coefficients ( $\rho$ ) and p-value in different vessels

| Vessels examined                                                 | n  | $\rho$ | p      | Publication |
|------------------------------------------------------------------|----|--------|--------|-------------|
| Superficial and deep leg veins                                   | 35 | 0.55   | <0.01  | I           |
| Deep and muscle leg veins                                        | 21 | 0.64   | <0.01  | I           |
| Superficial hand and foot veins in patients with previous DVT    | 46 | 0.77   | <0.001 | II          |
| Superficial hand and foot veins in patients without previous DVT | 22 | 0.67   | <0.01  | II          |
| Epigastric and iliac arteries of uraemic patients                | 46 | 0.35   | <0.05  | VI          |

A correlation was also found in the PA activity between various arteries and concomitant deep veins from all anatomic regions examined (VI).

PA activity in patients with overweight, diabetes mellitus, arteriosclerosis and uraemia

Table V shows the PA activity in arteries and veins in relation to weight and various diseases, where N indicate a PA activity which is not different from the activity in patients without the below mentioned diseases. The arrows indicate a decreased PA activity in the vessel wall compared with that of normal patients.

Table V

|                        | PA activity in |       | Publication |
|------------------------|----------------|-------|-------------|
|                        | arteries       | veins |             |
| Overweight $\geq 10$ % | N              | ↓     | I, VI       |
| Diabetes mellitus      | N              | N     | I, VI       |
| Arteriosclerosis       | ↓              | N     | VI          |
| Uraemia                | N              | N     | VI          |

PA activity and surgical trauma

The preoperative PA activity in superficial hand veins was abnormally low, i.e. below 6 arbitrary units in 6 out of 108 patients undergoing general major surgery and in 4 out of 56 patients undergoing prostatic surgery (III, IV). A postoperative decrease in the PA activity by more than 0.5 arbitrary unit was found in 90 (83 %) patients undergoing major general surgery (III), in 27 (75 %) patients undergoing transvesical prostatic surgery and in 6 (30 %) patients undergoing transurethral prostatic surgery (IV).

A postoperative decrease in PA activity to abnormally low values, i.e. below 6 arbitrary units was found in 44 (41 %) of the patients undergoing major surgery (III), in 18 (50 %) of the patients undergoing transvesical prostatic surgery (IV) and in 2 (10 %) of the patients undergoing transurethral prostatic resection (IV).

The PA decrease following major surgery was seen already at the end of the operation (III) and no further depression of the PA activity was found during the observed postoperative period. On the 14th postoperative day there was a normalization of the activity to preoperative values in 27 (77 %) patients (III).

#### PA activity and postoperative DVT

The  $^{125}\text{I}$ -fibrinogen uptake test revealed postoperative DVT in 5 out of 31 patients (III), all with a positive phlebography. Nine of the remaining patients (III, IV) had clinical signs of venous thrombosis and in 5 of them phlebography showed deep venous thrombosis. Gastro-intestinal surgery was performed in 7 of the patients with thrombosis (6 with malignant diseases, III) and transvesical prostatic surgery in the other 3 (IV). The preoperative PA activity was abnormally low in one of the patients who developed a postoperative DVT. Postoperatively 9 of the patients had abnormally low values (III, IV).

#### PA activity and risk factors predisposing to postoperative DVT

Preoperative PA activity, postoperative variations in PA and the number of patients with an abnormally low PA activity were independent of age, malignant disease, type of anaesthesia (general or epidural), peroperative blood loss, and postoperative infection (III, IV).

All overweight patients who underwent major general surgery had a postoperative PA decrease in superficial hand veins, whereas 60 % of normalweight patients had such a decrease (III). An operation lasting 180 min or more significantly increased the number of patients with a postoperative PA decrease (III).

There was a significantly higher frequency of patients with a postoperative PA decrease in those, where vascular surgery had been performed compared with the postoperative PA response in patients undergoing gastric surgery (III).

## Fibrinolysis, factor VIII and mechanical prophylaxis against DVT

Neither intermittent pneumatic compression (IPC) nor graduated compression stockings influenced plasma fibrinolytic activity, VIII:C or VIIIR:Ag in the compressed extremity (arm or leg) or in the general circulation. Compression stockings, however, were only used to the legs (V). Nor were there any variations in the plasma fibrinolytic activity, VIII:C or VIIIR:Ag in the treated leg during the various phases of the pump cycles (inflation period, deflation period and after finished compression) (V). There was no significant difference in the PA activity of superficial veins in the treated extremity compared with the activity of veins obtained from the contralateral side which was used as a control. The PA activity in hand veins was the same as the activity in foot veins (V). The results were independent of the intermittent compression pattern (quick or slow), pressure of the graduated stockings (18 or 35 mm Hg), the duration of the treatment, the extremity compressed (leg or arm) and sex.

## DISCUSSION

### Methodological aspects

Several different techniques are used to localize and to assess the PA activity in various tissues. Thus, using an extraction technique with high molar potassium thiocyanate Albrechtsen (1957) and using a histochemical technique Todd (1958) both demonstrated different fibrinolytic activity in various organs.

Kwaan & Astrup (1963, 1967b) using a modification of Todd's fibrin slide technique, determined the "focal lysis time", which is the shortest time of incubation to produce clearly visible lysis. By further modification Pandolfi et al (1967) could increase the accuracy of the PA assessment. With his technique not only the time of incubation, but also the size and shape of the lytic area were taken in account.

Other modifications of the fibrin slide technique have been described and have been used in other laboratories (Scott & Blaszcynski 1965, Fujinama & Gore 1967, Kester 1969, Constantini et al 1969b, Cunliffe et al 1975, Risberg et al 1975, Browse et al 1977a, Donner et al 1977, Monnens et al 1979).

In tissue cultures Åstedt & Pandolfi (1972), Pandolfi et al (1974) and Pandolfi & Åstedt (1977) demonstrated a technique which makes it possible to quantify the release of the fibrinolytic activators.

Johnson & Mansfield (1978) measured the PA content in veins by estimating the radioactivity released from a  $^{125}\text{I}$ -tagged fibrin clot by incubating that clot with a known weight of the vessel wall. This method, however, is not strictly reproducible and requires large specimens (Wu & Mansfield 1979).

Risberg & Claeson (1979) used a chromogenic substrate for quantitative determination of PA in tissues. With that technique the activators were extracted with potassium thiocyanate from sections of frozen tissues. The activators then reacted with the substrate and was measured photometrically. Using other synthetic substrates Huseby et al (1977) and Nieuwenhuizen et al (1977) described assays for determination of the PA activity.

Since activators now can be purified and differentiated from urokinase the vascular activator can be directly assessed in small quantities in plasma with an immunoradiometric technique (Holmberg et al 1982b). This method, however, does not measure the biologic activity.

The histochemical method used in this study presents several advantages. It requires a small amount of material, it provides information on the localization of the fibrinolytic activity and appropriately modified measures the strength of such activity. There are, however, some sources of error with this method, i.e. the freezing procedure of the specimens, the thickness of the sections, the fibrinogen used, the preparation of the fibrin film, the storage of the specimens and the temperature during the consolidation of the fibrin film (Pandolfi et al 1972).

In this study all sections were cut by the same laboratory assistant with a Harris' cryotome. By this procedure, the sections have uniform thickness which is important (Pearce & Marks 1974). The use of the same fibrinogen batch throughout the study is important since different batches gives different values for the fibrinolytic activity. To obtain a uniform thickness of the fibrin film fixed volumes of fibrinogen and thrombin were spread on a constant area of the slide and this preparation was always performed by the same laboratory assistant. It is also important that the temperature during the consolidation time of the fibrin slides is not too high. We found in our laboratory that a temperature of +19°C or below prevented an undesirable digestion of the fibrin slides.

It is important that biopsy specimens are obtained from the same localization when the activity is compared during various intervals from the same patient since the activity differ in different regions (Blatny 1966a, Pandolfi et al 1968, Büttner & Schmidt 1972, Wolfe et al 1979).

A good reproducibility of the method described by Pandolfi et al (1968) has been shown and now confirmed (see Table II). Peterson et al (1973) and Risberg (1976) both using a modification of Todd's fibrin slide technique also reported a good reproducibility.

Different criteria to express the fibrinolytic activity by different modifications of the histochemical technique do not allow direct numerical comparison between studies. With the technique described by Pandolfi et al (1967, 1968) the size of the vessel wall and the diameter of the specimens do not influence the results obtained in the scoring system (Wolfe et al 1979). This is in contrast to the technique described by Risberg (1976) where the quantitation of the fibrinolytic activity is based on the size of the lytic zones in relation to the diameter of the vessels.

In superficial limb veins the PA activity is mainly seen in the adventitia, where the vasa vasorum are localized and the intima of the vessel wall is usually inactive (Pandolfi et al 1967,



1968, 1969, Yao et al 1974). Others, however, have reported that detached endothelial cells from the intima are fibrinolytically active (Todd 1961, 1964a,b, Warren 1964). Detachment of cells can occur during the freezing and thawing procedure (Constantini et al 1969b). The appearance of fibrinolytic activity in the intima influences the scoring sum of the lytic zones, as the activity in the intima and the adventitia confluence with increasing incubation periods. This results in a higher scoring sum of the fibrinolytic activity on the fibrin films. In this study altogether 208 out 4580 fibrin slides showed a very high intimal activity and the PA activity in the vasa vasorum of the vessel wall was therefore difficult to analyse. Therefore all these fibrin slides were excluded.

In arteries there is little or no activity in the intima (Blatny 1966b, Yao et al 1974, Donner et al 1977). The main activity is localized to the adventitia like in veins. Anatomically Higginbotham et al (1963) described the distribution and function of the vasa vasorum, consisting of afferent and efferent vessels which supply the vessel wall. This helps to explain how the PA activity is emptied from the vasa vasorum into the lumen of the blood vessels where fibrinolysis exert its action on fibrin.

In another study Geiringer (1951) found no vascularization of the intima and inner portions of the media in healthy young human aortas, which explain the lesser activity seen in these vessels with histochemical technique.

In arteries Noordhoek-Hegt & Brakman (1974) showed inhibitors localized in the intima of arteries, whereas activators are localized to the adventitia of the vessel wall.

In lack of other more sensitive and quantitative methods to assay the fibrinolytic activity in the vessel wall, the fibrin slide technique is still the method of choice for determination of PA. The method has several advantages and a good reproducibility which as been discussed earlier. It should be emphasized that histochemical technique reflects the biologic activity whereas immunologic assays not do. The immunologic assay is not used

routinely at our laboratory yet, and that is the reason why histochemical method is used in this study.

#### PA activity in veins

The main sources of origin of postoperative DVT are the muscle veins (Kakkar et al 1969, Becker et al 1970, Nicolaides et al 1971, Nylander & Olivecrona 1976) and the soleus sinusoides (Flanc et al 1968, Negus et al 1968, Borow & Goldson 1981). Earlier investigations have shown that many patients with venous thrombosis have a decreased PA activity in superficial hand and/or foot veins (Pandolfi et al 1969, Isacson & Nilsson 1972, Nilsson & Isacson 1973, Browse et al 1977a, Jarrett et al 1977, Ciavarella et al 1979, Sundqvist et al 1981, Ljungnér et al 1982). In this study there was a correlation in the PA activity between superficial, deep and muscle leg veins (I) which is of importance since thrombi of clinical significance are localized to deep veins, but specimens are obtained from superficial veins. Moreover, a correlation in the PA activity between superficial hand and foot veins (II) implies that although thrombi usually occur in deep leg veins, a representative PA determination can be obtained from superficial hand veins. This is useful in clinical situations as it is technically easier to obtain specimens from hand veins than from foot veins and besides, it causes less discomfort to the patient. Infections are rare after biopsy from the hand.

In the present study the PA activity was the same in deep veins obtained above and below the knee, which is in contrast to the activity in superficial leg veins, where the activity is higher in the proximal part of the great saphenous vein than it is in the distal part (Blatny 1966a, Pandolfi et al 1968, Büttner & Schmidt 1972, Wolfe et al 1979). In deep veins Nicolaides et al (1976) found a lower PA activity in the soleus and popliteal veins compared with the activity in femoral veins. Their results, however, were based on 6 specimens obtained from cadavers. An important question in the present study was whether the PA activity in the leg veins might be affected by arterial disease (I) as all

patients underwent a leg amputation because of arterial insufficiency except one. An observation weighing against this was that the activity in superficial leg veins in this study agreed well with the activity in superficial leg veins in healthy controls, described by Pandolfi et al (1967, 1968, 1969).

From the literature there is an indication of a higher PA activity in arm veins than in leg veins (Pandolfi et al 1969, Isacson & Nilsson 1972, Wolfe et al 1979) but the specimens in these series were not systematically obtained from hand and foot veins respectively. According to the results of this study (II) there seems to be two groups of persons, one with the same PA activity in hand and foot veins and another with a higher activity in hand veins. This division was independent of whether or not, previous venous thrombosis had existed, but patients with earlier venous thrombosis had a significantly lower PA activity in foot veins than those without. None of the patients in this study were bed-ridden which is an important information as it is known that the fibrinolytic response after venous occlusion is the same in arms and legs when the patients are immobilized during a fortnight or more (Karaca & Nilsson 1971).

The local fibrinolytic response after venous occlusion in healthy volunteers is three to four times higher in an arm than it is in a leg (Robertson et al 1972a). This discrepancy in the results between the local plasma fibrinolytic activity after venous occlusion and the PA content of arm and foot veins (II) might indicate that the PA release mechanism after venous occlusion is weaker in the legs than it is in the arms.

#### PA activity in arteries

In the literature there are conflicting data about the arterial PA activity. This might be due to the fact that most studies are based on specimens obtained from cadavers. Little is known how the PA activity is influenced by the terminal disease, anoxia, chock, interval between death and necropsy and the storage of the cadaver.

In 5 series the arterial PA activity has been studied in living humans. Thus, Blatny (1966b) found that the activator activity of vessel walls assessed with an extraction technique was significantly higher in the adventitia than the activity in the intima which was practically inactive.

Yao et al (1974) found a reduced fibrinolytic activity in atheromatous arteries obtained during surgery.

Almér et al (1975) showed that the activity in temporal arteries was the same as the activity in superficial hand veins in diabetics, and furthermore, there was a correlation in the activity between these vessels. Median PA in temporal arteries in their study did not differ from the median PA in non-arteriosclerotic arteries in the present study (VI).

Pandolfi et al (1971) and Donner et al (1977) both demonstrated that the activity in the adventitia of arteries obtained during surgery did not differ significantly from the activity of corresponding vessels obtained at necropsy.

In the present study the arterial PA activity did not differ in specimens obtained from different anatomical regions, i.e. epigastric, pudendal and renal arteries in patients undergoing hernia repair surgery, varicose vein surgery and nephrectomy (living donors). Median PA was 7.0 (confidence interval at 95 % level, 3.5 - 10.0) arbitrary units. Thus, it can be concluded that median PA in arteries without arteriosclerotic lesions was the same as the median PA in superficial veins obtained from arm in healthy volunteers described by Nilsson & Pandolfi (1976).

Results from a few studies have indicated a higher PA activity in veins compared with that of arteries (Todd 1959, 1961 1964a, Pandolfi et al 1971) whereas others have found the same activity in arteries and veins (Almér et al 1975). In the present investigation arteries and concomitant veins had the same PA activity in all regions examined with one exception. The activity in epigastric veins was significantly higher than in corresponding arteries both in patients undergoing a hernia repair surgery and in uraemic patients. The reason for this discrepancy in the

activity is unclear.

#### PA activity in patients with overweight

It is known that obese patients have a lower PA activity in superficial hand veins than normal weight patients (Almér & Janzon 1975). Furthermore, earlier data have indicated that there is an inverse correlation between the degree of overweight and the fibrinolytic activity determined in blood (Bennett et al 1966) and in vein walls (Almér & Janzon 1975). It can be concluded from this study that overweight patients had a lower PA activity not only in superficial leg veins but also in deep leg veins (I). Furthermore, all overweight patients who underwent major surgery had a postoperative PA decrease in superficial hand veins compared with 60 % of normal weight patients (III).

#### PA activity in patients with diabetes mellitus

The reason for the discrepancy between the findings of the same PA activity in patients with and without diabetes mellitus in superficial and deep veins in the present investigation (I, VI) and the observation of a decreased PA activity in superficial hand veins in diabetic patients as described by Almér & Nilsson (1975) is unclear. In their study about one fourth of the diabetic patients had a lower activity in superficial hand veins. Most of their patients, however, were overweight, a factor which can contribute to the low activity (see discussion above). The difference between the results of this study compared with data produced by Almér & Nilsson (1975) can not be explained by the severity of the disease, as our patients had a terminal uraemia caused by diabetes and the uraemic diseases did not influence the PA activity (VI). It is known that diabetics with retinopathy have a higher PA activity in superficial hand veins than diabetics without retinopathy (Almér et al 1975b) which promote the results in this study.

### PA activity in patients with uraemia

Various uraemic diseases did not influence the PA activity in arteries or in veins (VI), a finding which is in agreement with the results from 5 patients described by Isacson & Nilsson (1969). In their study the PA activity in superficial hand veins obtained on the day before dialysis was the same as the activity in healthy volunteers. It is known that uraemic patients have a low circulating fibrinolytic activity (Edwards et al 1964, McNicol et al 1965, Sharma et al 1967) supposed to be due to an increased concentration of urokinase inhibitors or a defect release mechanism of the PA activity (Isacson & Nilsson 1969, Larsson 1971, Hedner & Nilsson 1971, reviewed by Nilsson 1980). Besides, in a recent study Canavese et al (1982) showed that haemodialysis can correct and eliminate inhibitors of the fibrinolytic system.

### PA activity in patients with arteriosclerosis

There are conflicting data in the literature about the arterial fibrinolytic activity in patients with arteriosclerosis. Thus, Onoyama & Tanaka (1969) and Constantini et al (1972) found a low PA activity in atherosclerotic arteries obtained in cadavers. These data have also been confirmed by Yao et al (1974) in patients undergoing surgery because of arterial insufficiency. On the other hand, Astrup & Coccheri (1962), Kwaan & Astrup (1967a) and Donner et al (1977) found an increased activity in atherosclerotic arteries obtained at necropsy.

There is also conflicting data about the plasma fibrinolytic activity in arteriosclerotic patients. Thus, a low activity has been found in both venous and arterial samples (Nestel 1959, Naimi et al 1963, Peabody et al 1964, Fearnley et al 1967, Chakrabarti et al 1968), whereas a normal fibrinolytic activity has been reported by Nilsson et al (1980a).

In the present study the PA activity was lower in atherosclerotic leg arteries obtained below the knee in patients undergoing a leg amputation because of arterial insufficiency than the activity in nonatherosclerotic arteries obtained above the knee (VI). It



should be pointed out, that all the leg arteries except three were occluded. This finding can not be explained in favour of the localization from where the arteries were obtained as the activity in various arteries obtained from different anatomical regions was found to be the same. The finding of a low PA activity in leg arteries obtained from patients with arterial insufficiency might perhaps due to the fact that such vessels have a defect synthesis of PA in the vessel wall. This suggestion is further strengthened by the finding of a low PA activity in 28 atherosclerotic aortas obtained during surgery (Ljungrén et al, unpublished observations).

From the results in this study and data obtained in the literature it is still a matter of debate whether or not the low PA activity in arteriosclerotic patients is an effect of the disease.

#### Surgical trauma and PA activity in superficial hand veins

Surgical trauma is followed by an increase of several coagulation factors i.e. factors II, VII, VIII and X (Egeberg 1962, Amundsen et al 1963, Ygge 1970), fibrinogen (Godal 1962, Korvald et al 1974) and fibrinogen/fibrin degradation products (Cash et al 1969, Korvald et al 1974). There is also a raised level of inhibitors of the fibrinolytic system (Tsitouris et al 1961, Innes & Sewitt 1964, Griffiths et al 1977b). Antithrombin III and plasminogen concentration are usually decreased (Korvald et al 1974, Jørgensen et al 1980, Seyfer et al 1981, Lindström 1982). Recently, Hedner et al (1982) found a parallel decrease of factor XII and a fibrinolytic inhibitor of the plasminogen activation in patients undergoing surgery. Trauma also causes an activation of the coagulant activity of platelets (Walsh et al 1976). These changes have been reviewed by Brozovic (1977) and are assumed to contribute to postoperative venous thrombosis. During and/or immediately after major surgery and major trauma there is a rise in plasma fibrinolytic activity, followed by a period of marked suppression known as the "fibrinolytic shut down" (Bergentz & Nilsson 1961, Ygge 1970, Mansfield 1972, Gordon-Smith et al 1974, Knight et al 1977, Griffiths et al 1977a).

With histochemical technique Larsson & Risberg (1977) showed that cellular injury is accompanied by a local release of the PA from tissues. It has been shown that major surgery is followed by a decrease of the PA content in vein walls as seen by histochemical technique (Åberg et al 1974, Isacson & Ljungnér 1978) and by a defect release of the activators after venous occlusion (Rawles et al 1975, Åberg & Nilsson 1978, Griffiths 1979).

There is a lack of systematic studies of the postoperative changes in PA activity after surgical trauma but from the present study it can be concluded that the PA decreases in superficial hand veins appeared intraoperatively in the majority of patients and that no further depression was seen during the observed postoperative period. On the 14th postoperative day the PA activity had returned to the preoperative value in 77 % of the patients (III).

The mechanism of an intra- or postoperative PA decrease in the vessel wall is unknown. Alternative explanations are conceivable. It may be an effect of a defect synthesis of activators in the vessel wall. A decreased PA content in the vessel wall might also be an expression of still unknown inhibitors.

It is known that stimuli such as anxiety and stress of a patient cause a release of activators from the vessel wall to the blood (Macfarlane & Biggs 1946, Chakrabarti et al 1969, Prentice et al 1972, Britton et al 1974, Cash 1975). Release of activators in the vessel wall has been found in association with anoxia (Clarke & Cliffton 1962). In their study the local fibrinolytic activity in the blood after venous occlusion was directly related to the fall in the oxygen tension during a manoeuvre.

There are inhibitors of the fibrinolytic system localized to the arterial as well as to the venous vessel walls (Noordhoek-Hegt & Brakman 1974) but their role in association with surgery are unknown. Taken all these theories together it is probable that it is a combination of the above mentioned mechanisms that is responsible to the low PA content in superficial veins, which is seen after surgery.

The postoperative "fibrinolytic shut down" in plasma may be an expression of the decreased postoperative PA content in the vessel wall as a result of a defect synthesis and/or release of the activators but can also be due to the increased levels of inhibitors of the fibrinolytic system which is seen in plasma after surgery (Tsitouris et al 1961, Innes & Sewitt 1964, Bennett 1967, Gallus et al 1973, Rennie et al 1974, Griffiths et al 1977b).

The duration of the postoperative PA decrease in the vessel wall is of the same length as the period when a decreased circulating fibrinolytic activity has been observed (Wuelfing & Brandau 1968, Britton et al 1974). As known, Robertson et al (1972b) found an increased circulating fibrinolytic activity after single venous occlusion, but repeated stimulations did not give any further response until after an interval of 11-14 days. Also prolonged physical activity causes partial depletion of endothelial stores of PA, which is seen as low plasma fibrinolytic activity (Keber et al 1979).

In an experimental study in pigs Wu & Mansfield (1980) demonstrated that trauma is followed by a marked suppression of the circulating fibrinolytic activity which is correlated with the PA decrease in the great saphenous vein. This response was seen immediately after the operation and lasted to the end of the observation period, 9 days. In a rat study Risberg & Bylock (1981) using histochemical technique demonstrated that endothelial injury to veins was followed by a significantly PA decrease already one hour after the trauma. At the time of re-endothelialization three weeks later, there was a return of PA activity to pre-injury values in the vessel wall.

The immediate PA decrease after surgical trauma in the vessel wall (III) coincides with the time when several factors of pathogenic importance for the development of postoperative venous thrombosis are changed and when the blood flow of the leg veins is reduced (Browse 1962, Doran et al 1964, Clark & Cotton 1968, Lindström et al 1977). The intra and/or early postoperative period is the time when most DVT occur (Coon & Collier 1959, Flanc et al 1968, Lambie et al 1970, Kakkar et al 1970, Hartsuck & Greenfield 1973, Nicolaidis 1973, Borow & Goldson 1981). It has

been pointed out that two factors in the Virchow's triad have to coexist before local thrombosis can occur (reviewed by Wessler & Gitel 1981).

A postoperative decrease in the PA activity was a common finding in the present study in most patients who underwent major surgery and after transvesical prostatectomy which is in agreement with the findings presented by Åberg et al (1974), Åberg & Nilsson (1978) and Isacson & Ljungnér (1978). Only 30 % of patients undergoing transurethral surgery had a postoperative decrease in the PA activity. Thus, differences in the fibrinolytic decrease may be one of several factors which can explain why postoperative DVT is less common after TURP than after TVP (Mayo et al 1971, Sripad et al 1971, Kakkar 1972, Nicolaides et al 1972b, Hedlund 1975, v.Hospenthal et al 1977).

In the present study all patients subjected to TURP had a duration of the operation of 60 min or less and TURP technique was therefore defined as minor surgery. According to some workers minor surgery is not accompanied by any alteration in the postoperative plasma fibrinolytic activity (Olow 1963, Mansfield 1972, Griffiths 1979) but these results are in contrast with the findings of others (Andersson et al 1962, Knight et al 1977). The incidence of postoperative DVT is significantly lower in patients undergoing minor surgery than in those subjected to major surgery (Flanc et al 1969, Kakkar et al 1970, Hartsuck & Greenfield 1973, Sigel et al 1974).

#### PA activity and postoperative venous thrombosis

An abnormally low PA activity in superficial hand veins and/ or an impaired fibrinolytic release after venous occlusion are common findings in patients with recurrent venous thrombosis (Isacson & Nilsson 1972, Nilsson & Isacson 1973, Ciavarella et al 1979, Ljungnér et al 1982). The significance of a defect fibrinolytic system as one of several important factors in the development of postoperative DVT has been stressed by several authors (Tsapogas et al 1971, Mansfield 1972, Sautter et al 1973, Knight

et al 1977, Comp et al 1979, Fahmy et al 1981, Hirsh 1981) whereas others have found it of less importance (Flute et al 1972, Korvald et al 1974, MacIntyre et al 1976, Browse et al 1977b, Lindström 1982). The role of an abnormally low PA activity in the vessel wall after surgical trauma in the pathogenesis of postoperative DVT is unknown.

Owing to lack of facilities screening for postoperative DVT with <sup>125</sup>I-fibrinogen uptake test (FUT) was only possible in a subgroup (Table VI). This group consisted of 31 patients who underwent gastro-intestinal major surgery (III), 5 of whom had a positive uptake test. Among the rest of the patients DVT was suspected from clinical signs in 9 patients, all of them underwent phlebography and 5 had thrombosis (III, IV). It is well known that FUT is a sensitive, accurate and simple method, whereas clinical diagnosis of postoperative DVT is a very insensitive procedure (reviewed by Gallus 1976).

Table VI

The postoperative PA activity in 31 patients screened with <sup>125</sup>I-fibrinogen uptake test (FUT)

| The postoperative decrease<br>in PA activity (arb.units)<br>to values | FUT pos | FUT neg | No. of patients |
|-----------------------------------------------------------------------|---------|---------|-----------------|
| <6 units                                                              | 4       | 13      | 17              |
| ≥6 units                                                              | 1       | 13      | 14              |
|                                                                       | 5       | 26      | 31              |

An abnormally low postoperative PA activity was found in 44 out of 108 (41 %) patients undergoing major surgery (III), in 18 out of 36 (50 %) patients undergoing TVP and 2 out of 20 (10 %) patients undergoing TURP (IV). All patients received low dose heparin (III, IV) or dextran (III) and altogether postoperative venous thrombosis was found in 10 patients, 9 of whom had an abnormally low postoperative PA activity. It should be mentioned that this is not the true incidence of postoperative venous thrombosis, since only 31 patients were screened with the FUT test. Only one of the patients in the thrombosis group had an abnormally low preoperative PA activity which indicates that determination of the individual preoperative PA activity in the vessel wall seems to be of little value as a predictor of postoperative DVT.

Plasma fibrinolytic activity also seems to be a poor screening test for patients with a high risk for postoperative venous thrombosis (Gallus et al 1973, Reilly et al 1980). Furthermore, in a prospective study Becker (1972) failed to find any correlation between the degree of stasis-elicited blood fibrinolysis and the occurrence of postoperative venous thrombosis.

Summing up, the results in this study indicated that although an abnormally low postoperative PA activity was a common finding after surgery only one fourth of patients with an abnormally low PA activity developed thrombosis in the FUT group. On the other hand, 9 out of 10 patients with postoperative DVT had an abnormally low postoperative PA activity.

#### PA activity and risk factors for postoperative DVT

Several risk factors are considered to predispose to postoperative DVT such as age, body weight, duration of the operation and malignant disease (Kakkar et al 1970, Rokoczi et al 1980, Borow & Goldson 1981). Other factors such as peroperative blood loss (Lahnborg & Bergström 1975, Ishak & Marley 1981), postoperative infection (Nicolaidis et al 1972a, Fossard et al 1974, Törngren et al 1980) and the anaesthetic technique (Davis et al 1980, Thorburn et al 1980, Hendolin et al 1981, Modig et al 1981) have



been stressed too. These risk factors have been reviewed by Hume et al (1970) and Nicolaides & Irving (1975).

Among all of the above mentioned factors, age is probably the most important single factor. But, from this study, it can be concluded that age did not influence the PA activity in superficial hand veins (III), whereas Johnson & Mansfield (1979) found a lower PA activity in the great saphenous vein with increasing age.

The number of patients with a postoperative PA decrease was more frequent when the duration of the operation lasted 180 min or more than in those with a shorter duration (III). With an increasing duration of the operation, however, the blood loss tends to increase. But, when the peroperative blood loss was analysed as a single factor it did not influence the results (III).

The proportion of patients with a postoperative decrease in the PA activity was the same in patients with malignant and benign diseases (III) which is in agreement with the findings of Browse et al (1977b), Åberg & Nilsson (1978) and Ljungnér & Bergqvist (1981). Also plasma fibrinolytic response after venous occlusion is the same in patients with and without malignant diseases (Rennie & Ogston 1975). Thus, the increased frequency of postoperative DVT in patients operated for malignant diseases does not seem to due to a low PA content or a defect release mechanism of activators in the vessel wall. On the other hand, some workers have found a pronounced postoperative "fibrinolytic shut down" after surgery in association with malignant diseases (Allenby et al 1973, Griffiths 1979, O'Brien et al 1979) which can be explained by the high levels of fibrinolytic inhibitors described in patients with malignant diseases (Soong & Miller 1970).

Postoperative infection according to the criteria given in publication III did not influence the pre- or postoperative PA pattern compared with the PA activity in patients without any infection (III).

In a few studies, it has been shown that plasma fibrinolytic

response is altered after a standard operation with different techniques of anaesthesia. Thus, Rem et al (1981) and Simpson et al (1982) both found that general anaesthesia but not epidural or spinal analgesia caused a depression of plasma fibrinolytic activity. From the present study it can be concluded that there was no differences between a postoperative PA activity and anaesthetic techniques (IV). It should be stressed, however, that the specimens were obtained from superficial hand veins.

Thus, of all risk factors analysed in this study only weight and the duration of the operation seemed to significantly influence the number of patients with a postoperative PA decrease in superficial hand veins.

#### Fibrinolysis, factor VIII and mechanical thromboprophylactic methods

Different opinions exist whether intermittent pneumatic compression (IPC) stimulates the fibrinolytic system. Allenby et al (1973), Knight & Dawson (1976) and Tarney et al (1980) all found an increased fibrinolytic activity determined as euglobulin clot lysis time (ELCT) after finished IPC when blood samples were obtained before and immediately after compression. Allenby et al (1973) and Tarney et al (1980) used compression of the leg and found a fibrinolytic enhancement in the general circulation. These results are in contrast to those of Robertson et al (1972b) who found that venous occlusion increased the local fibrinolytic response but had no effect on the systemic circulation.

ECLT is a simple though somewhat crude screening method (Nilsson et al 1978) where the patients own fibrinogen is used as a substrate. It should be stressed that the results obtained with this method are influenced by the alterations in plasma fibrinogen which occur postoperatively (Hickman et al 1973, Korvald et al 1974). The fibrin plate method, in which exogenous fibrinogen is added, was used in the present study (V) and the results are in agreement with those of O'Brien et al (1979) and Moser et al (1981), who found no significant fibrinolytic activation after

IPC.

Allenby et al (1973) and Knight & Dawson (1976) investigated surgical patients before and after compression with IPC and blood samples were thus obtained after the surgical trauma. This may explain the discrepancy between their results and those observed in the present study where the blood samples were obtained after a compression of at least two hours with IPC and 24 hours with the stockings respectively. The compression period, however, was finished before the surgical procedure, except in those 4 patients, where a catheter was placed in the femoral vein during the operation.

Not only the fibrinolytic system was examined but also VIIIR:Ag and VIII:C (V). VIIIR:Ag has been identified in the endothelial cells (Bloom et al 1973, Hoyer et al 1973) and is also synthesized there (Jaffe et al 1973, Holmberg et al 1974). Various stimuli such as adrenalin, nicotinic acid and vasopressin or its analogue DDAVP cause a rapid increase of both factor VIII and the circulating fibrinolytic activity (Nilsson et al 1970, Mannucci et al 1975). After venous occlusion or infusion of DDAVP there is a release of not only PA content from the vessel wall but also VIIIR:Ag in parallel (Nilsson et al 1980b, Nilsson et al 1982). The results of an unchanged fibrinolytic activity after IPC and graduated stockings respectively in the present study are therefore supported by the results of VIIIR:Ag, which was not significantly increased.

As for the PA determination in hand and foot veins after compression and static compression stockings respectively the contralateral extremity was used as a control. None of the patients had any visible varicosities, signs of superficial or deep venous insufficiency in the control leg.

The fibrinolytic response after venous occlusion is dependent on the duration of the occlusion period (Tighe & Swahn 1963) and the occlusion pressure. Amery et al (1962) found a weak fibrinolytic response after venous occlusion with a low pressure. To obtain an optimal fibrinolytic response the pressure should be between systolic and diastolic levels and with an occlusion period of 20

min (Amery et al 1962, Robertson et al 1972b). This pressure, however, is painful to the patient and is impossible to use for longer periods in connection with IPC treatment.

In an experimental study in pigs, IPC with a pressure of 225 mm Hg significantly lowered the PA activity in the great saphenous vein examined with histochemical technique compared with the activity in control animals (Wu & Mansfield 1979). In humans it is also known that surgery on an extremity in "bloodless field" using a tourniquet with a pressure of about 450 mm Hg is accompanied by a local release of PA from the tissues (Larsson & Risberg 1977). Complete circulatory arrest of a limb is followed by an increase of the local fibrinolytic response in the occluded limb (Kwaan & McFadzean 1956, Nakahara & Sakahashi 1967) and also in the systemic circulation (Klenerman et al 1977, Fahmy et al 1981).

Using IPC as a thromboprophylactic method Ah-See and co-workers (1976) have demonstrated that an inflation pressure of 40 mm Hg is followed by optimal changes of the blood flow and this pressure is the most effective to use in preventing postoperative DVT.

One explanation why IPC and graduated static compression is ineffective in stimulating the release of PA as well as factor VIII might thus be the low pressure used. It can be concluded that the well documented effect of mechanical methods against postoperative DVT is not an effect of any stimulation of the fibrinolytic system. The mechanism of the thromboprophylactic effect is to be given by other explanations (Bergqvist et al 1982).

## CONCLUSIONS

This study warrants to the following conclusions

- 1 a) The PA activity in superficial leg veins was significantly lower than the activity in deep and muscle veins obtained from the same level. The PA activity in muscle and deep veins did not differ significantly. The activity in deep veins was the same in the proximal and distal part of the leg, whereas the activity in the great saphenous vein was higher above the knee than it was below. There was a correlation in the PA activity between superficial and deep leg veins.
  - b) The PA activity in superficial hand veins was the same as the activity in superficial foot veins in 57 % of the patients and higher in the hand veins in 38 %. A correlation was found in the PA activity between hand and foot veins. These results were independent of the existence of previous deep venous thrombosis. The activity was significantly lower in foot veins in patients with previous thrombosis compared with the activity in corresponding foot veins in patients without thrombosis.
  - c) The PA activity in arteries and concomitant veins obtained from different regions did not differ significantly except for the epigastric vessels, where the activity was significantly higher in the veins. There was a correlation in the PA activity between different arteries and concomitant veins in all regions examined.
- 2) Overweight patients had a lower PA activity in superficial and deep leg veins than patients of normal weight. Arterial PA activity was uninfluenced by the weight. Diabetes mellitus and terminal uraemia did not influence either arterial or vein wall PA activity. The PA activity in atherosclerotic arteries in patients undergoing a leg amputation was

significantly lower than the activity in arteries without atherosclerosis. The activity in leg veins in patients with arterial insufficiency was uninfluenced by the arteriosclerotic diseases.

- 3) The PA activity was significantly decreased in superficial hand veins immediately after major surgery, and lasted to the 14th postoperative day, when the activity had returned to the preoperative value in 77 % of the patients. A postoperative PA decrease by more than 0.5 arbitrary unit was found in 83 % of patients undergoing major surgery, in 75 % of patients undergoing transvesical prostatic surgery and in 30 % of patients undergoing transurethral prostatic resection.
- 4) An abnormally low postoperative PA activity was a common finding in superficial hand veins in patients without venous thrombosis screened with the  $^{125}\text{I}$ -fibrinogen uptake test. On the other hand, practically all patients with verified thrombosis had an abnormally low postoperative PA activity. Of all risk factors analysed, obesity and a prolonged duration of the operation significantly influenced the number of patients with a decreased postoperative PA activity.
- 5) Intermittent pneumatic compression and graduated static compression did not influence either circulating fibrinolytic activity and factor VIII locally or in the general circulation. Nor was there any alterations in the PA activity in the vessel wall after the two compression techniques.



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COMPARISON BETWEEN THE PLASMINOGEN ACTIVATOR ACTIVITY IN WALLS  
OF SUPERFICIAL, MUSCLE AND DEEP VEINS

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ABSTRACT

Determinations were made of the plasminogen activator activity (PA) of biopsy specimens of the great saphenous vein and at the same level of the femoral or the popliteal vein in 35 patients undergoing amputation of the leg because of arterial insufficiency and in one because of deep venous insufficiency. The PA was measured with a modification of Todd's fibrin slide technique. In 21 patients the PA was also determined in biopsy specimens of a muscle vein at the same level of the leg. The PA of the walls of superficial veins proved significantly lower than that of the walls of deep major veins. No difference in activity was demonstrable between muscle veins and deep major veins. A significant correlation was found between the PA of superficial and deep veins.

INTRODUCTION

It is known that deficiency of plasminogen activator activity (PA) is a contributory cause of deep venous thrombosis (DVT) (1-4). It is assumed that a deficiency of activity of PA in the deep veins also means a deficiency in the superficial veins. Investigation of patients with so-called spontaneous DVT therefore often includes determinations of the PA in a biopsy specimen of a superficial vein. As far as we know, the PA in superficial veins has never been compared directly with that in deep veins. Muscle veins are reportedly the source of origin of DVT (5, 6). The aim of this study was therefore to compare the PA of biopsy specimens of superficial veins with that of deep major veins and muscle veins at corresponding levels of legs undergoing amputation.

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Key words: Plasminogen activator activity, superficial veins, deep major veins, muscle veins

## MATERIAL AND METHOD

The clinical material consists of twenty 28-91 year old men (median age 77) and fifteen 48-97 year old women (median age 77) undergoing above-knee (9 patients) or below-knee (26 patients) amputation of the leg. In 34 patients, including one with B rger's disease (man, aged 38), the indication for the amputation was arterial insufficiency. In one patient, a 28 year old man, amputation was done because of deep venous insufficiency which had developed after trauma (Table I). Sixteen patients had diabetes mellitus. Of these, 7 were receiving insulin, and the other 9 oral drugs. By the time of operation, 33 patients had gangrenous ulcerations of the foot and/or lower leg. Six patients were more than 10 % overweight. Two of the patients had earlier had DVT, verified by phlebography; none of the other patients had any history of DVT. All the operations were done under spinal anesthesia.

The biopsy specimens, 0.5-1.0 cm long, were excised peroperatively from the great saphenous vein (35 patients), the femoral (9 patients) or popliteal vein (26 patients), at the same level. In 21 patients a biopsy specimen was also obtained at the same level of a muscle vein, and in 8 from a superficial vein on the back of the hand.

TABLE I

Indication and Level of Amputation in 35 Patients

| Level      | Arterial<br>insufficiency | Arterial insufficiency<br>and diabetes mellitus | Deep venous<br>insufficiency |
|------------|---------------------------|-------------------------------------------------|------------------------------|
| Below knee | 13                        | 12                                              | 1                            |
| Above knee | 5                         | 4                                               |                              |

### Determination of the PA of vein walls

The biopsy specimens were hermetically enclosed and frozen at  $-70^{\circ}\text{C}$ . They were afterwards cut into  $0.8\text{ }\mu\text{m}$  sections, which were incubated at  $37^{\circ}\text{C}$  in close contact with a thin fibrin film rich in plasminogen in a moist atmosphere in an incubator. The film was obtained by coagulation of plasminogen-rich fibrinogen with thrombin. On incubation the plasminogen is activated to plasmin with the appearance of a zone of fibrinolysis around the tissue. By incubation for a varying period it is possible to estimate the fibrinolytic activity in arbitrary units ranging from 0 to 12. The method is a modification of Todd's technique (7, 8) and has earlier been described in detail (9). The reproducibility of the method is very good (10).

### Statistical methods

Mann Whitney rank sum test (11) and Student's t-test were used. The correlation coefficients were calculated according to Spearman's rho ( $\rho$ ) (11). The differences between the results obtained in the various groups were considered to be significant when  $p < 0.05$ .

## RESULTS

The median value for all the superficial veins was 4.0 arbitrary units (confidence interval at 95 % level 2.5-5.0). The corresponding value for deep major veins was 6.0 (5.0-6.5) and for the muscle veins 5.5 (4.0-6.0) arbitrary units.

In 9 patients the leg was amputated above the knee. The PA in superficial venous system of the thigh in these patients was higher than that in the lower leg of patients with a below-knee amputation, 5.0 and 2.75 arbitrary units, respectively. The corresponding values for the deep venous system were 6.0 and 5.5 arbitrary units (Table II). In one patient the venous system was examined both above and below the knee. Below the knee the activity was 1.5 units in the superficial venous system and 1.0 in a deep major vein. At later reoperation because of arterial insufficiency of the stump the activity was 5.5 in the great saphenous vein and 6.0 arbitrary units in the femoral vein.

The median value of the activity of the specimens from the hand was 7.5 (6.5-9.0) arbitrary units (Table II).

Sixteen patients had diabetes mellitus. The median value of the superficial venous system in this group was 4.5 units and for the deep major veins 6.0 arbitrary units. The corresponding values for non-diabetics were 4.0 and 5.5 arbitrary units.

Two patients had previously had DVT, for which they had been treated with ethylestrenol for at least one year before the operation. The PA in the great saphenous vein was 7.5 and 8.0 arbitrary units, respectively. The corresponding values for the deep major vein were 5.5 and 8.0 units, respectively.

TABLE II

Number (n), Median Value (m) and Respectively First and Third Quartile (q<sub>1</sub>, q<sub>3</sub>) of the PA in the Venous Systems Below and Above Knee and in Superficial Veins of the Hand

| Biopsy specimen          | n  | Below knee<br>PA in m (q <sub>1</sub> , q <sub>3</sub> ) | n | Thigh<br>PA in m (q <sub>1</sub> , q <sub>3</sub> ) |
|--------------------------|----|----------------------------------------------------------|---|-----------------------------------------------------|
| Superficial vein         | 26 | 2.75 (1.5 - 5.5)                                         | 9 | 5.0 (3.5 - 5.5)                                     |
| Deep major vein          | 26 | 5.5 (5.0 - 6.5)                                          | 9 | 6.0 (4.0 - 6.0)                                     |
| Muscle vein              | 21 | 5.5 (4.0 - 6.0)                                          |   |                                                     |
| Superficial hand<br>vein | 8  | 7.75 (6.5 - 9.0)                                         |   |                                                     |

Six patients were overweight and the median value of the PA in the superficial veins was 2.25 (1.0-4.5) and the corresponding value for major veins 4.75 (2.5-6.5) arbitrary units.

The PA in the wall of superficial veins of the lower leg proved significantly lower than that of deep veins ( $t = 2.3732$ ,  $p < 0.01$ ). The PA of the wall of deep major veins was not found to differ significantly from that of muscle veins at corresponding levels of the leg ( $p > 0.6$ ).

Fig. 1 shows a significant correlation between the PA of the superficial and deep venous system in the same patient ( $P = 0.55$ ,  $p < 0.01$ ), and Fig. 2 between the deep veins and muscle veins ( $P = 0.64$ ,  $p < 0.01$ ).

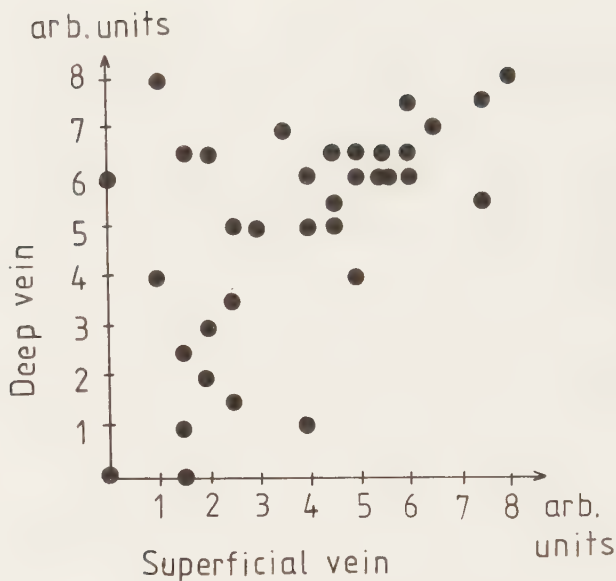


FIG. 1

Plasminogen activator activity (PA), in arbitrary units, of walls of superficial and deep veins at corresponding levels in 35 patients. Biopsy specimens obtained at amputation of the leg. Correlation coefficient 0.55.

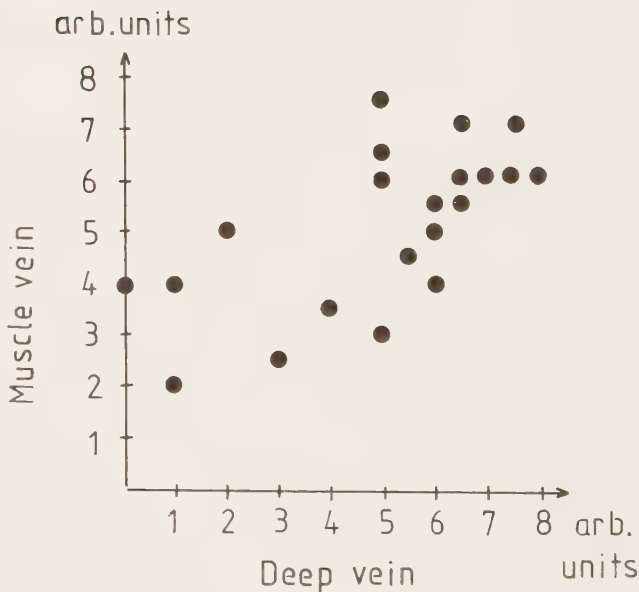


FIG. 2

Plasminogen activator activity (PA), in arbitrary units, of walls of deep veins and muscle veins at corresponding levels in 21 patients. Biopsy specimens obtained at amputation of the leg. Correlation coefficient 0.64.



## DISCUSSION

This investigation shows a significantly lower PA of the walls of the superficial veins than of the deep major veins at a corresponding level of the leg and, secondly, a significant correlation between the two systems. An important question is whether the PA of biopsy specimens of deep veins might be affected by the patients' arterial disease. An observation weighing against this being so was that the values found by us agreed well with those found by Pandolfi and co-workers (3,4,9) in healthy controls.

Biopsy specimens of the superficial and deep veins to be compared must be taken from corresponding levels of the vascular system, since the PA of the great saphenous vein for example, is higher at the level of the groin than it is below the knee (9,12,13). In 9 of our patients the leg was amputated above the knee and the median value of the PA of the biopsy specimens of superficial veins was higher than that of such veins below the knee.

As known (9), the PA of the walls of superficial veins in the back of the hand is significantly higher than that in veins on the dorsal part of the foot. As biopsy specimens were obtained from only 8 hand veins, the values found were not studied for any correlation.

According to other investigations (14,15), the PA of the superficial venous system is abnormally low in about one fourth of all diabetics. But this was not found to be so in our material. Diabetics and non-diabetics were therefore pooled in the different analyses.

In the 2 patients previously treated with ethylestrenol for at least one year, the PA was high in the superficial as well as in the deep venous system, an observation in line with others (16) that ethylestrenol raises the PA of the veins in patients with a defective fibrinolytic system.

The PA of both the superficial and the deep venous system in the 6 overweight patients was lower than that in the remaining 29. This is in agreement with the finding that the PA of superficial hand veins is significantly more often low in obese than in non-obese subjects, both in diabetics and in non-diabetics (15).

Muscle veins have narrower lumina than corresponding superficial and deep veins. There is, however, no differences in PA between a biopsy specimen of the great saphenous vein and that of a collateral at the same level of the leg despite the difference in diameter between them (13). In other words, the sum of arbitrary units of the PA of a vessel wall does not vary with the diameter of the vessel.

No difference in PA of biopsy specimens was found between the deep veins and the muscle veins at corresponding levels of the leg, while as pointed out earlier, that of the superficial veins was lower. A significant correlation in PA was found between deep veins and muscle veins. Using a modification of Todd's fibrin slide technique (17) Nicolaides (18) studied the focal lysis time of deep veins and soleus veins in 6 cadavers and found the activity to be lower in the soleus veins than in the popliteal vein and the femoral vein. The focal lysis time is the minimum incubation time of a segment of a vein necessary to show a clearly outlined area of lysis in the active zones of the sections (grade I of fibrinolysis according to Pandolfi's modification of Todd's histochemical method). The focal lysis time is held to be an uncertain indicator of the fibrinolytic activity in tissue (9). In addition, in Nicolaides' investigation the biopsy specimens were not obtained at corresponding levels of the leg.

In conclusion, our investigation has shown that the PA of the walls of superficial veins is lower than that of the deep venous system at the corresponding level. There is a significant correlation between the superficial, muscle and deep veins.

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## COMPARISON OF THE PLASMINOGEN ACTIVATOR ACTIVITY OF SUPERFICIAL HAND AND FOOT VEINS

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### ABSTRACT

The plasminogen activator (PA) activity in superficial hand and foot veins was determined with a fibrin slide technique in 68 patients (46 with and 22 without thrombosis). The activity in the hand veins was identical to that of the foot veins in 57 % of the patients and higher in 38 %. There was a correlation between activity in hand and foot veins in patients with and without thrombosis. Thus, biopsy specimens from superficial hand veins can be used for assessing the PA activity in patients with leg thrombosis.

### INTRODUCTION

A deficient fibrinolytic activity in the vein walls is an important contributory cause of venous thrombosis. An abnormally low plasminogen activator (PA) activity has been reported in superficial arm and in leg veins in patients with leg thrombosis (1, 2). In patients with idiopathic venous thrombosis Isacson and Nilsson (3) found that the PA activity in arm vein and that in an ipsilateral leg vein were always either normal or abnormal in both. In healthy subjects Nilsson and Isacson (4) found a higher PA activity in arm than in leg veins.

Although thrombosis is most common in the deep veins of the leg, biopsy specimens for assessing the patients' PA activity are usually obtained from a superficial hand vein. It is known that there is a correlation of the PA activity between the superficial and deep leg veins (5), but nothing is known about the correlation of the PA activity in superficial veins from the hand and the foot. Nor is it known whether the PA activity in hand veins is representative for the activity in leg veins in patients with deep venous thrombosis of the legs.

The aim of this study was therefore to compare the PA activity in superficial hand and foot veins in patients with and without thrombosis.

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Key words: plasminogen activator activity, fibrinolytic activity, deep vein thrombosis, hand veins, foot veins

## CLINICAL MATERIAL

Sixty-eight patients, 24 females, aged 13-69 (median 34) and 44 males, aged 18-71 (median 35) were examined. Seventeen patients were above 50 years and 11 patients were at least 10 % overweight (6). Forty-six patients had a history of venous thrombosis; 24 of them had 2-5 episodes, the last one at least 3 months before this investigation. All cases of thrombosis were confirmed phlebographically and localized to the legs. Thirty-four patients had been treated with oral anticoagulants until two days before this investigation. The non-thrombotic group consisted of 22 patients. Seven of them underwent varicose vein surgery, but were otherwise healthy, and the remaining 15 patients had a familial predisposition of venous thrombosis.

## METHOD

### PA determination

One biopsy specimen of a superficial hand vein and one of a superficial foot vein were obtained at the same time under local anaesthesia (Lidocain-Chloride 0.5 %). In patients undergoing varicose vein surgery the specimens were obtained under general anaesthesia. In patients with venous thrombosis and in those undergoing varicose vein surgery the foot specimen was always obtained from the leg on the unaffected side. Hand and foot specimens of a given patient were always removed from the same side.

The PA activity in the vessel wall was measured using Todd's (7) fibrin slide technique, as modified and described in detail by Pandolfi et al. (8). This method is semiquantitative and the PA activity is expressed in arbitrary units. The normal range for hand veins in our laboratory is 6-10 arbitrary units.

The reproducibility of the method is good (9). A difference in the PA activity between hand and foot veins exceeding 0.5 arbitrary unit was considered significant.

### Statistical calculations

The correlation coefficient was calculated according to Spearman's rho ( $\rho$ ). Mann-Whitney rank sum test, Student's t-test, Chi-Square test and the Sign test were used when differences were calculated (10). Differences were accepted as significant when  $p < 0.05$ .

## RESULTS

The median PA in superficial hand veins was 6.5 (2.5-9.5) arbitrary units and 6.5 (1.0-9.5) in superficial foot veins. There was no difference in the PA activity between hand and foot veins in 39 patients, 30 with and 9 without thrombosis (Fig. 1). A difference of 1.0 arbitrary unit or more (range 1.0-3.0) between hand and foot veins was found in 29 patients, including 13 without thrombosis (Fig. 1). Of these 29 patients, 26 had a higher PA activity in the hand veins ( $p < 0.01$ , Table I). Thus 26 out of 68 (38 %) had a higher PA activity in the hand veins. When there was a difference in the PA activity between the hand and foot veins, patients with thrombosis had a lower activity in the foot veins than those without ( $p < 0.05$ ). The results were independent of age and sex.

TABLE I

Number and Distribution of Patients with a Positive or a Negative Difference in the PA Activity ( $>0.5$  arb.unit) between Hand and Foot Vein (a Positive Difference means a Higher PA Activity in Hand Veins than in Foot Veins)

|                       | Number of patients with: |                       | p        |
|-----------------------|--------------------------|-----------------------|----------|
|                       | A positive difference    | A negative difference |          |
| Total material        | 26                       | 3                     | $< 0.01$ |
| Patients with DVT     | 14                       | 2                     | $< 0.01$ |
| Patients without DVT  | 12                       | 1                     | $< 0.01$ |
| Age $>50$ years       | 8                        | 0                     | $< 0.01$ |
| Age $<50$ years       | 18                       | 3                     | $< 0.01$ |
| Female                | 13                       | 1                     | $< 0.01$ |
| Male                  | 13                       | 2                     | $< 0.01$ |
| Overweight patients   | 3                        | 2                     | N.S.     |
| Normalweight pateints | 23                       | 1                     | $< 0.01$ |

TABLE II

Correlation Coefficient ( $\rho$ ) between Superficial Hand and Foot Veins and p Values in the Whole Patient Group and in Subgroups

|                                 | Number of patients | $\rho$ | p        |
|---------------------------------|--------------------|--------|----------|
| Total material                  | 68                 | 0.73   | $<0.001$ |
| Patients with venous thrombosis | 46                 | 0.77   | $<0.001$ |
| Patients without thrombosis     | 22                 | 0.67   | $<0.01$  |
| Age $>50$ years                 | 17                 | 0.83   | $<0.001$ |
| Age $<50$ years                 | 51                 | 0.73   | $<0.001$ |
| Female                          | 25                 | 0.78   | $<0.001$ |
| Male                            | 43                 | 0.78   | $<0.001$ |
| Overweight patients             | 11                 | 0.65   | $<0.05$  |
| Normalweight patients           | 51*                | 0.75   | $<0.001$ |

\* information lacking in 6 patients

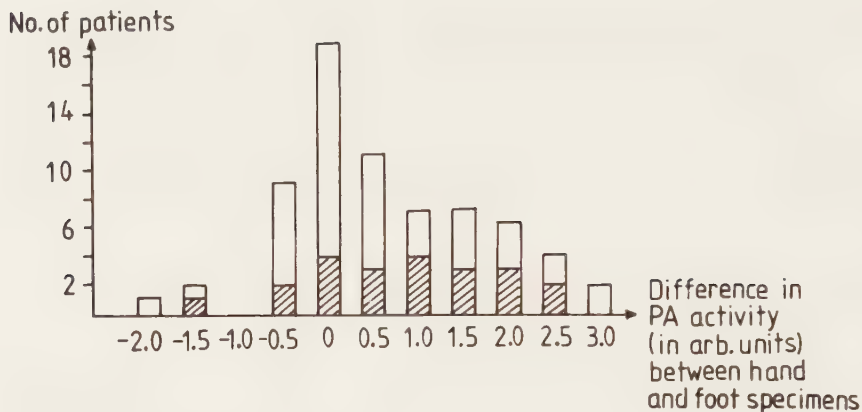


FIG. 1

The difference in PA activity in arbitrary units between hand and foot veins. The shaded segments of the columns denote 22 patients without thrombosis. The blank area denotes 46 patients with leg thrombosis.

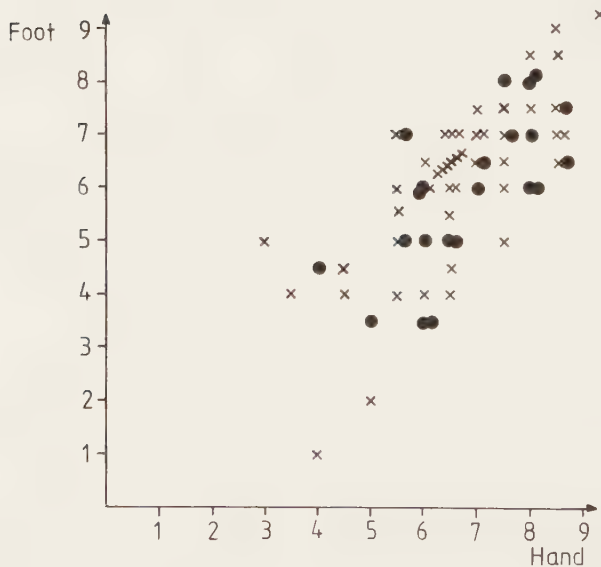


FIG. 2

The PA activity in arbitrary units in 68 patients (● denotes 22 patients without thrombosis). Biopsy specimens obtained from superficial hand and foot veins. Correlation coefficient 0.73 ( $p < 0.001$ ).



The PA activity of hand and foot veins did not vary with the side from which the specimen had been obtained, nor between patients taking and not taking oral anticoagulantia.

A correlation was found between the hand and the foot vein activity in the whole material ( $P=0.73$ ,  $p<0.001$ , Fig. 2). As seen from Table II, a correlation between hand and foot veins was also found in the subgroups divided with respect to age, sex, weight and the presence or absence of thrombosis.

## DISCUSSION

This study was undertaken to ascertain whether there was any correlation between PA activity of superficial hand and foot veins. Such a correlation was indeed found in the total study group and all the subgroups investigated.

After venous occlusion of the arms and the legs, Robertson et al. (11) found a correlation between circulating fibrinolytic activity of the arms and legs in healthy volunteers. There is evidence that the fibrinolytic activity in plasma originates from tissue activators, i.e. the PA in the vessel wall (12, 13).

The finding of a correlation in the PA activity between hand and foot veins is of practical importance since it implies that though thrombi occur in the legs, biopsy specimens for determination of PA activity can be obtained from superficial hand veins. It is technically easier to obtain a vein specimen from the hand than from the foot. Besides, biopsy from a hand vein causes less discomfort and is less often followed by infection.

One group, consisting of 39 patients (57 %) had the same PA activity in the hand as in the foot veins, the other consisting of 29 patients exhibited different activity. The activity was higher in hand veins than in foot veins in 26 of them. The distribution into 2 groups was independent of the thrombotic disease, age, sex and weight. According to Karaca and Nilsson (14) patients confined to bed have roughly the same plasma fibrinolytic activity after venous occlusion of the arm as after occlusion of the legs. However, none of our patients in whom the PA activity in the foot veins was identical with or higher than that in the hand veins were bedridden.

In healthy volunteers Robertson et al. (11) found that the fibrinolytic activity in the blood is four times higher after venous occlusion in the arms than in the legs.

Although not systematically studied before, there are several investigations indicating a higher PA activity in the arm than in the leg vein; in patients with thrombosis (1, 2), in healthy subjects (8), in patients with varicose vein disease (15), in arteriosclerotic patients (5) and in a post-mortem material (16). Our observations of a subgroup, where the majority of the patients indeed had the same activity in the hand and the foot veins has never been reported before.

Judging from the observations made in this study, the PA activity in hand veins was identical with that in foot veins in 57 % of the patients, and higher in 38 %. There was a correlation between hand and foot veins in patients with as well as without thrombosis.

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THE INFLUENCE OF MAJOR SURGERY ON PLASMINOGEN ACTIVATOR ACTIVITY  
IN SUPERFICIAL HAND VEINS

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**ABSTRACT.** In 108 patients the plasminogen activator (PA) activity in superficial hand veins was measured with a fibrin slide technique before and after a major surgical operation. In the early postoperative period the PA activity was significantly decreased and the PA activity did not return to its preoperative value until the 14th postoperative day. The postoperative decrease was more than 0.5 arbitrary unit in 90 patients, resulting in an abnormally low value in 44. Overweight or an operation time of 180 min or more was found to increase the proportion of patients with a postoperative decrease in the PA activity. No sure relationship was demonstrable in the postoperative PA activity between patients with venous thrombosis and those without.

Immediately after major trauma or major surgery there is a rise in the circulating fibrinolytic activity followed by a fall to subnormal values (2, 4, 9, 10, 11, 18, 21, 22, 26, 34, 35). Based on small series it has been claimed that surgical trauma also is accompanied by an early postoperative decrease in the plasminogen activator (PA) activity in superficial hand veins (1, 15). It is not known, however, when the decrease in the PA activity appears in relation to surgery or how long it lasts.

Patients with recurrent idiopathic venous thrombosis have an increased incidence of abnormally low PA activity in superficial veins of the arm and of the leg (14), but the significance of the PA activity for the development of postoperative deep venous thrombosis (DVT) has neither been evaluated. Nor is it known how the postoperative response in the PA activity in superficial hand veins is influenced by factors predisposing to DVT.

The aim of this study was to assess the postoperative changes in PA activity. The pre- and postoperative PA activity was related to factors which are considered to predispose to postoperative venous thrombosis and in a subgroup also to the development of DVT.

Table I. Operations and diagnoses in 108 patients

|                    | Diagnosis | Number |
|--------------------|-----------|--------|
| Gastric surgery    | benign    | 24     |
|                    | malignant | 13     |
| Colon surgery      | benign    | 5      |
|                    | malignant | 49     |
| Pancreatic surgery | benign    | 2      |
|                    | malignant | 5      |
| Vascular surgery   |           | 10     |

## MATERIAL

The material consisted of 108 patients, 37 women, aged 52-85 (median age 70) and 71 men, aged 31-91 (median age 67), all undergoing major general surgery (table I) under general anaesthesia. Prophylaxis against postoperative DVT was given to all the patients.

Thirteen patients (8 men) were at least 10 % overweight (3). The peroperative blood loss was estimated by the anaesthetist, based on the amount of blood found in the suction bottle system and in swabs, which was 100-7000 (median value 600) ml. The duration of operation was 65-720 (median value 160) min. Postoperative infection defined as pus in the wound, postoperative abdominal abscess, peritonitis or septicaemia was diagnosed in 32 patients.

## METHOD

### PA determination

A superficial vein segment 0.5-1.0 cm long was always obtained from the back of the hand under local anaesthesia (Lidocain-Chloride 0.5 %) and examined histochemically with Todd's (32) fibrin slide technique as modified and described in detail by Pandolfi et al. (27). According to this semiquantitative method, the PA activity is expressed in arbitrary units. The normal range in our laboratory is 6-10 arbitrary units. The reproducibility of the method is good (28). The PA activity was considered to have decreased when the difference between two estimations exceeded 0.5 arbitrary unit.

One biopsy specimen was always obtained preoperatively. A second specimen was obtained within the third postoperative day in all the patients except in 3 where this specimen was obtained between days 6 and 13. In 25 of the patients the second specimen was obtained at the end of the operation, from the hand on the side where no infusions had been given and no recording made of the blood pressure. A third specimen was obtained from 6 patients on the first postoperative day, from 31 between postoperative day 3 to 5, and later in the postoperative period in the others according to fig. 1. The three specimens from each patient were always analyzed by one person (H.L.) without any knowledge about the moment when they were obtained and the preparations of the fibrin film was always performed by the same laboratory assistant (I.v.H.).

Table II. Distribution of predisposing factors and percentage of patients in whom the PA fell postoperatively by more than 0.5 arb. unit

|                                      | Number of patients | Percentage of patients with a decrease ( $>0.5$ arb.unit) in PA activity within the third post-operative day | p       |
|--------------------------------------|--------------------|--------------------------------------------------------------------------------------------------------------|---------|
| Patients with age $<70$              | 70                 | 66                                                                                                           | N.S.    |
| Patients with age $\geq 70$          | 38                 | 63                                                                                                           |         |
| Patients with overweight             | 13                 | 100                                                                                                          | $<0.05$ |
| Patients with normal weight          | 95                 | 60                                                                                                           |         |
| Peroperative blood loss $<500$ ml    | 46                 | 67                                                                                                           | N.S.    |
| Peroperative blood loss $>500$ ml    | 62                 | 61                                                                                                           |         |
| Duration of operation $<180$ min     | 58                 | 52                                                                                                           | $<0.01$ |
| Duration of operation $\geq 180$ min | 50                 | 78                                                                                                           |         |
| Postoperative infection              | 32                 | 69                                                                                                           | N.S.    |
| No infection                         | 76                 | 63                                                                                                           |         |
| Patients with malignant disease      | 67                 | 63                                                                                                           | N.S.    |
| Patients with benign disease         | 41                 | 66                                                                                                           |         |

#### $^{125}\text{I}$ -labelled fibrinogen uptake test

Thirty-one patients (7 women and 24 men) aged 41-81 (median age 65) undergoing abdominal surgery were screened for postoperative DVT with the  $^{125}\text{I}$ -labelled fibrinogen uptake test for one week. When the isotope test was positive with an uptake increase of at least 20 % at two consecutive examinations, the patients were examined phlebographically which invariably confirmed the diagnosis of DVT.

#### Factors considered to predispose to postoperative venous thrombosis

Age, weight, peroperative blood loss, duration of operation, postoperative infection and surgery for malignant disease was related to the PA activity pre- and postoperatively.

The investigation was approved by the Ethical Committee, University of Lund, and all patients gave their written informed consent.

Table III. Age, sex, diagnosis, blood loss, duration of operation and the pre- and postoperative PA activity in arbitrary units in 7 patients with post-operative DVT

| Age | Sex | Operations and diagnoses       | Perop blood loss (ml) | Duration of op (min) | PA in arb. units |        |
|-----|-----|--------------------------------|-----------------------|----------------------|------------------|--------|
|     |     |                                |                       |                      | preop            | postop |
| 61  | M   | Gastric surgery (malignant)    | 910                   | 410                  | 7.0              | 5.5    |
| 65* | M   | Colon surgery (benign)         | 250                   | 115                  | 7.5              | 6.5    |
| 56* | M   | Pancreatic surgery (malignant) | 400                   | 135                  | 10.5             | 5.5    |
| 70* | M   | Colon surgery (malignant)      | 1200                  | 205                  | 7.0              | 3.0    |
| 47* | M   | Colon surgery (malignant)      | 7000                  | 300                  | 6.5              | 5.5    |
| 65* | M   | Colon surgery (malignant)      | 700                   | 220                  | 6.0              | 4.0    |
| 78  | M   | Colon surgery (malignant)      | 600                   | 165                  | 8.0              | 1.5    |

\* positive fibrinogen uptake test

Statistical methods

Chi-Square test (Yate's correction), Mann-Whitney rank sum test; Wilcoxon's rank sum test of paired observations; Student's t-test. A difference was considered significant when  $p < 0.05$ .

RESULTS

PA activity

Preoperatively the median PA was 8.0 (3.5 - 10.5) arbitrary units. In 6 of the patients the preoperative PA values was abnormally low, i.e. below 6 arbitrary units. None had a history of previous deep venous thrombosis (DVT). The individual PA activity in arbitrary units is given in fig. 1. By the end of the operation the PA activity had fallen by more than 0.5 arbitrary unit in 17 of 25 (68 %) patients. The median PA activity at this time was 6.5 (2.5 - 9.0) arbitrary units ( $p < 0.01$ , fig. 1). A postoperative decrease in the PA activity was noted in 90 patients, in 44 of whom the activity fell to an abnormally low value at the time for the second specimen. Eight patients out of 35 (23 %) still had a decreased PA activity on the 14th postoperative day but only one had an abnormally low value. The median PA at this time had reached the preoperative value 8.0 (5.5 - 10.0) arbitrary units.

Factors considered to predispose to thrombosis

Preoperative PA activity, postoperative pattern of the PA or the frequency of an abnormally low PA activity was not influenced by age, peroperative blood loss, postoperative infection or malignant disease (table II).

Table IV. The postoperative PA activity in 31 patients screened with  $^{125}\text{I}$  fibrinogen uptake test (FUT)

|                                             | FUT pos | FUT neg |    |
|---------------------------------------------|---------|---------|----|
| A decreased PA activity to<br><6 arb. units | 4       | 13      | 17 |
| A decreased PA activity to<br>≥6 arb units  | 1       | 13      | 14 |
|                                             | 5       | 26      | 31 |

In all overweight patients the PA activity fell by more than 0.5 arbitrary unit within the third postoperative day, but only in 60 % in normal weight patients ( $p<0.05$ , table II).

In patients who had undergone an operation lasting 180 min or more, a postoperative decrease in the PA activity was seen significantly more often than in those subjected to a shorter operation ( $p<0.01$ , table II). All 12 patients with an operation duration of 300 min or more showed a postoperative decrease.

The postoperative PA response did not differ between different types of surgery, except in the group of patients undergoing vascular surgery. The postoperative PA activity in this group was lower than in patients undergoing gastric surgery ( $p<0.05$ ). The median duration of the operation was 315 (190-720) min in the former group, compared with 150 (65-410) min in the latter ( $p<0.01$ ).

### Venous thrombosis

Five out of 31 patients screened for DVT showed a positive fibrinogen uptake test and all these had a postoperative decrease in the PA activity (table III), in 4 of them to an abnormally low value. In 13 out of 26 patients with a negative fibrinogen uptake test the postoperative PA activity was abnormally low. No significant difference was found in patients with a positive or negative fibrinogen uptake test and an abnormally low PA activity (table IV). In two of the remaining 77 patients, DVT was suspected on clinical grounds and confirmed phlebographically. There was a postoperative decrease in the PA activity to an abnormally low value in these two patients (table III).



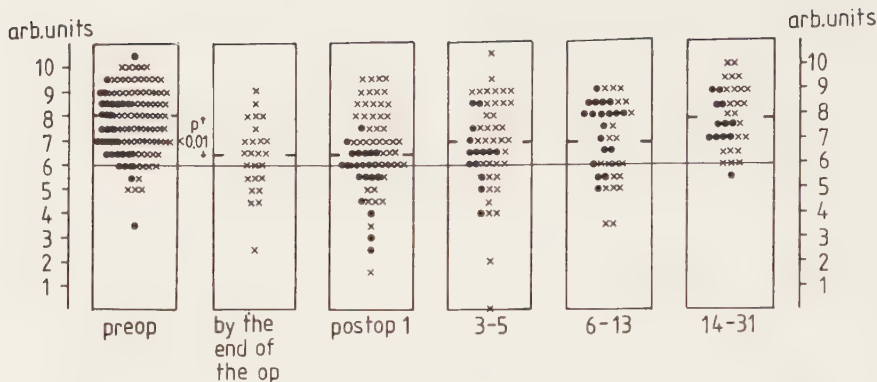


Fig. 1. PA activity in arbitrary units in superficial hand veins in 108 patients. Biopsy specimens obtained preoperatively and twice postoperatively. • denote patients with  $^{125}\text{I}$ -labelled fibrinogen uptake test. The horizontal line denote the lower normal limit for PA.

## DISCUSSION

The etiology of postoperative venous thrombosis is multifactorial. One factor of possible importance is an alteration in the fibrinolytic system. A rapid decrease in PA seems to be the pattern of the PA response to major surgery. Åberg and Nilsson (1) found a significant decrease in the PA activity on the third postoperative day in 22 patients undergoing surgery because of rectal carcinoma. The PA decrease coincides with the time when the blood flow in leg veins is reduced, which is the period when most postoperative thrombi debute (5, 12, 17, 19, 24). Thus two factors of importance in the pathogenesis of thrombosis are influenced in the very early postoperative period.

A decrease of the postoperative PA activity to abnormally low values is a common finding in patients undergoing major surgery, but only 4 out of 17 patients with an abnormally low PA activity screened with  $^{125}\text{I}$ -fibrinogen uptake test developed postoperative DVT. The role of a low PA activity in the pathogenesis of DVT is well known from patients with idiopathic venous thrombosis (14). A low PA activity also seems to be a risk to develop postoperative DVT but not as a single factor. Determination of the preoperative PA activity in patients undergoing surgery seems to give little information on the individual risk for postoperative DVT. Nor can determination of postoperative PA differentiate between patients who do and do not develop postoperative DVT.

Many risk factors are of importance for the development of postoperative thrombosis, one of them being advanced age (5, 8, 17, 25, 29). Preoperative median PA was independent of age. Nor did the postoperative decrease in PA vary with age, whereas Johnson and Mansfield (16), found a significant decrease in the PA activity in the great saphenous vein with increasing age.

Overweight patients is a risk group for postoperative DVT (5, 13, 17). In our study these patients invariably developed a decrease in postoperative PA.

The duration of operation influenced the postoperative PA response. With increasing duration of an operation, however, the blood loss tends to increase, but we found no effect of the amount of blood loss itself on the postoperative

PA activity. The longer the operation the greater the risk of postoperative DVT (5, 30, 31).

Postoperative infection is another factor predisposing to DVT (7, 23, 33) but this complication did not influence the postoperative median PA activity. Neither did the PA response vary with the type of disease, i.e. malignant or benign, for which the patients were operated upon. This is in accord with the finding by Browse et al. (6) that malignant disease is not accompanied by any changes in the tissue fibrinolytic activity. Neither Åberg and Nilsson (1), nor Ljungnér et al. (20) found any difference in the PA activity in superficial veins of the hand between patients with malignant and non-malignant disease.

Summarizing, a decrease in the PA activity of superficial hand veins was found in the early postoperative period in most patients subjected to major surgery. The PA activity did not return to its preoperative value until the 14th postoperative day. A postoperative decrease in the PA activity was seen significantly more often in overweight patients and in patients undergoing an operation lasting 180 min or more.

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PLASMINOGEN ACTIVATOR ACTIVITY IN PATIENTS UNDERGOING TRANSVESICAL AND  
TRANSURETHRAL PROSTATECTOMY

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ABSTRACT

In 36 patients undergoing transvesical prostatectomy (TVP) and in 20 undergoing transurethral prostatic resection (TURP) the plasminogen activator (PA) activity was determined in superficial hand veins according to a fibrin slide technique preoperatively and on the first postoperative day. In the TVP group there was a significantly larger proportion of patients with a postoperative PA decrease than in patients undergoing transurethral surgery. This finding was independent of anaesthetic technique (general or epidural) and the duration of the operation.

Postoperative deep venous thrombosis (DVT) is more common after transvesical prostatectomy (TVP) than after transurethral prostatic resection (TURP) (1,2,3,4). This may be explained, at least partly, by technical differences between the two methods.

A defective fibrinolytic system is an important causal factor of venous thrombosis. An abnormally low plasminogen activator (PA) content in the superficial hand veins is often found in patients with recurrent venous thrombosis (5).

After major surgery there is a "fibrinolytic shut-down" in the circulation, but not after minor surgery (6,7,8,9,10,11). Major surgery is also followed by a decrease in the PA activity in superficial hand veins (11,12,13). It is not known, however, whether there is any difference between the postoperative PA response to TVP and to TURP that could help to explain the difference in the frequency of postoperative DVT.

The aim of this study was to compare the postoperative PA response in patients undergoing surgery with the TVP and TURP technique and to elucidate the influence of different anaesthetic techniques on the postoperative PA response in superficial hand veins.

Material and Method

The material consisted of 56 patients, 36 operated upon with TVP and 20

with TURP because of hyperplasia of the prostate. Table I summarizes some characteristics of the two groups. Twelve patients were at least 10 % overweight according to the criteria of Bengtsson et al. (14). General anaesthesia (barbiturates, halothane and nitrogen oxide) included muscle relaxants, Mepivacain-Chloride was used when epidural or spinal analgesia was given. Forced diuresis was not used in the postoperative period. All patients were given low dose heparin prophylactically against postoperative DVT. One gram of tranexamic acid was given i.v. preoperatively and 1 g three times a day postoperatively until the macroscopic haematuria had disappeared.

#### PA Determination

A biopsy specimen was obtained under local anaesthesia (Lidocain-Chloride 0.5 %) from a superficial hand vein preoperatively and on the first postoperative day. The PA activity was assessed histochemically with Todd's (15) fibrin slide technique, as modified and described in detail by Pandolfi et al. (16). According to this semiquantitative method, the fibrinolytic activity is expressed in arbitrary units. The normal range for superficial hand veins in our laboratory is 6-10 arbitrary units. The reproducibility of the method is good (17). The PA activity was considered to have decreased when the difference between two assessments exceeded 0.5 arbitrary unit.

Fully informed consent was given by the patients.

#### Statistical Calculations

The results were analyzed with Wilcoxon's rank sum test of paired observations, Mann-Whitney's rank sum test and Fisher's exact test. Statistical significance was considered when  $p < 0.05$ .

#### Results

##### TVP group

Two patients had an abnormally low PA activity preoperatively, i.e. below 6 arbitrary units (fig. 1) and one of them developed postoperative venous thrombosis. The preoperative median PA was 7.5 (5.0-10.0) arbitrary units.

A postoperative decrease in the PA activity by more than 0.5 arbitrary unit was found in 27 (75 %) out of 36 patients, in 18 of them to an abnormally low value (fig. 1). The median PA on the first postoperative day had fallen to 6.0 (2.5-8.5) ( $p < 0.01$ ). The proportion of patients with a postoperative PA decrease was independent of the type of anaesthesia used (general or epidural) and the duration of the operation (table II).

##### TURP group

Two patients had an abnormally low PA activity preoperatively. The preoperative median PA was 8.0 (3.0-9.0) arbitrary units, which was not different from the preoperative value in the TVP group. Six (30 %) patients showed a postoperative decrease in the PA activity, two of whom to an abnormally low value (fig. 2). The median PA on the first postoperative day was 7.0 (2.5-9.0)



Table I. Distribution of factors that possibly capable of influencing the PA activity in superficial hand veins in patients subjected to transvesical and transurethral prostatectomy

| Surgical technique    | No. of patients | Age           | Over-weight | Type of anaesthesia |    | Duration of op (min) |
|-----------------------|-----------------|---------------|-------------|---------------------|----|----------------------|
| Trans-vesical (TVP)   | 36              | 72<br>(56-85) | 8           | General             | 13 | 80 (40-100)          |
|                       |                 |               |             | Epidural            | 23 | 60 (30-220)          |
|                       |                 |               |             |                     |    | ↑                    |
|                       |                 |               |             |                     |    | **                   |
|                       |                 |               |             |                     |    | ↓                    |
| Trans-urethral (TURP) | 20              | 72<br>(53-85) | 4           | Epidural            | 10 | 30 (20-50)           |
|                       |                 |               |             | Spinal              | 10 | 30 (15-60)           |

\*\* =  $p < 0.01$

Table II. Percentage of patients in the TVP and TURP group with a post-operative decrease in the PA activity of more than 0.5 arbitrary unit

|                          | TVP group       |                                              | TURP group      |                                              | Differences between the TVP and the TURP group<br>p |
|--------------------------|-----------------|----------------------------------------------|-----------------|----------------------------------------------|-----------------------------------------------------|
|                          | No. of patients | Percentage of patients with a decrease in PA | No. of patients | Percentage of patients with a decrease in PA |                                                     |
| Total                    | 36              | 75                                           | 20              | 30                                           | <0.01                                               |
| Anaesthesia epidural     | 23              | 73                                           | 10              | 30                                           | <0.05                                               |
| general                  | 13              | 76                                           | -               | -                                            |                                                     |
| Duration of the op (min) | ≤60             | 68                                           | 20              | 30                                           | <0.05                                               |
| >60                      | 17              | 82                                           | -               | -                                            |                                                     |

PA activity in  
arb. units postop

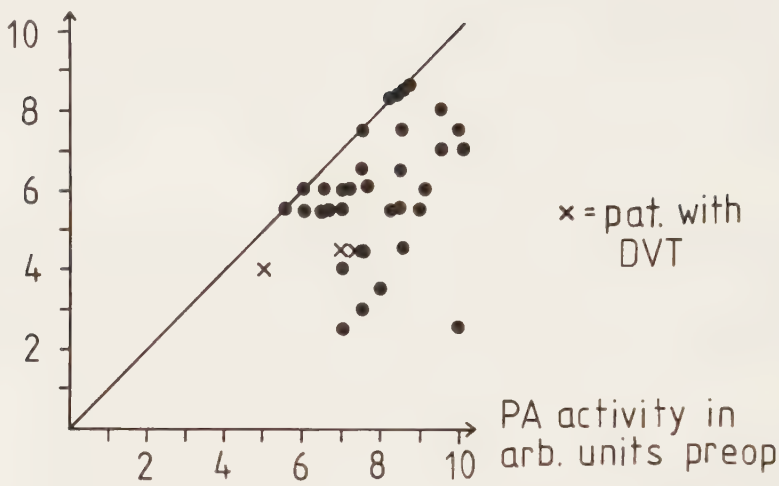


Fig. 1 Pre- and postoperative PA activity (arbitrary units) in patients undergoing transvesical prostatectomy.

PA activity in  
arb. units postop.

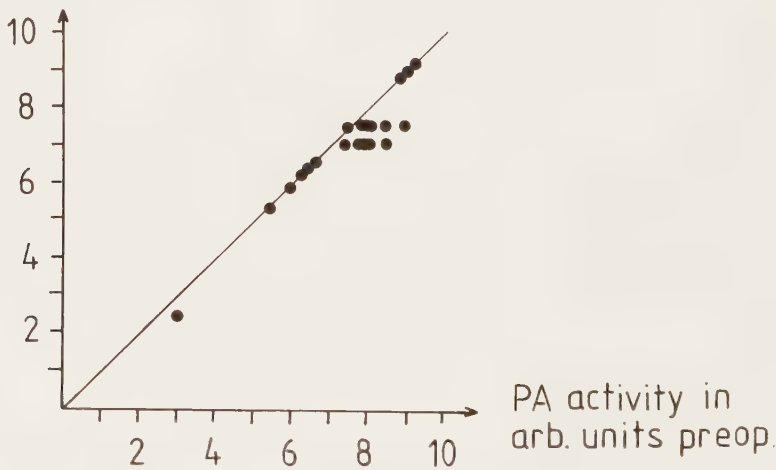


Fig. 2 Pre- and postoperative PA activity (arbitrary units) in patients undergoing transurethral resection of the prostate.

arbitrary units ( $p>0.05$ ). No difference in the postoperative PA response was found between 10 patients undergoing surgery under epidural and 10 under spinal analgesia.

#### Comparison of the PA Activity Between the TVP and the TURP Groups

As there was no difference between patients under epidural and spinal analgesia in the TURP group data from these two groups were pooled. The age and the frequency of overweight patients was virtually equal in both groups (table I). In the TVP group 75 % of the patients showed a postoperative decrease in PA activity, compared with 30 % in the TURP group ( $p<0.01$ , table II). This difference was independent of the anaesthetic technique and duration of the operation (table II).

#### Deep Venous Thrombosis

Three patients in the TVP group had clinical signs of DVT, which was phlebographically confirmed. All had an abnormally low PA activity on the first postoperative day (fig. 1).

#### Discussion

It is well established that postoperative DVT is less common after transurethral than after transvesical prostatectomy. Many possible explanations can be offered, such as differences in anaesthetic techniques, peroperative blood loss, duration of the operations, severity of the operative trauma, flow characteristics due to peroperative position, response of the fibrinolytic and coagulation systems to trauma and the time required for full postoperative mobilization.

This study concerned one of the factors, i.e. the postoperative PA response in superficial hand veins in patients undergoing TVP or TURP. A decrease in the postoperative PA activity by more than 0.5 arbitrary unit was found on the first postoperative day in 75 % of the patients undergoing transvesical surgery, compared with only 30 % in the TURP group.

It is known that a local liberation of the fibrinolytic activity from the prostatic tissue occurs during surgery. This can be counteracted by tranexamic acid, which does not, however, influence the PA activity in superficial hand veins (18).

In the TVP group the postoperative PA activity in superficial hand veins did not differ between patients undergoing surgery under general or epidural anaesthesia; nor was there any difference in the TURP group between patients under epidural or spinal analgesia.

As seen from table I, transvesical surgery lasted significantly longer than transurethral surgery. The proportion of patients with a postoperative decrease in PA was significantly higher in the TVP group than in the TURP group also when cases with an operation time of one hour or less were compared. This difference in the postoperative PA pattern between these two groups in this study can probably not be attributed to differences in the duration of the operation. On the other hand a relationship between the duration of the operation and the postoperative PA response has been observed in patients undergoing major general surgery lasting 180 minutes or more (13).

The difference in the proportion of patients with a postoperative decrease in PA can theoretically depend on differences in peroperative blood loss. However, the proportion of patients with a postoperative decrease in PA is not influenced by the amount of blood loss (13).

This study was performed without screening for postoperative venous thrombosis. Three patients had clinically diagnosed and phlebographically confirmed DVT of the leg. They all belonged to the TVP group and in all the PA activity fell to an abnormally low value on the first postoperative day. Such a decrease in the PA activity in superficial hand veins is a common finding in patients with postoperative venous thrombosis (11, 12, 13) and in many cases this decrease is seen already immediately after the operation (13) which is the time when most cases of postoperative DVT appear after transvesical prostatic surgery and after major surgery (1, 19, 20, 21, 22, 23).

In summary, the investigation showed that the proportion of patients with a postoperative decrease in PA was significantly larger after transvesical than after transurethral surgery. The postoperative fall in PA activity did not vary with the type of anaesthesia or the duration of the operation. It is therefore tempting to assume that the difference in the postoperative PA response between transvesical and transurethral surgery might be at least a contributory cause of the difference in frequency of postoperative DVT with the type of surgery.

#### Acknowledgements

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## EFFECT OF INTERMITTENT PNEUMATIC AND GRADUATED STATIC COMPRESSION ON FACTOR VIII AND THE FIBRINOLYTIC SYSTEM

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**Abstract.** Intermittent pneumatic compression (IPC) or graduated static compression stockings, both effective mechanical methods in the prophylaxis of postoperative deep venous thrombosis, have been investigated in 50 patients regarding the effect of compression on the fibrinolytic system and factor VIII activators. All the patients were subjected to one of the two methods before operation upon varicose veins. In 38 patients IPC with a pressure of 40 mmHg was applied on one leg or arm for at least two hours. In 23 of these patients the compression pattern was slow, two minutes' inflation and two minutes' deflation period. In the remaining 15 patients the compression pattern was quick, three seconds' inflation, followed by 20 seconds' deflation, three cycles a minute. Twelve patients were treated with a compression stocking on one leg for about 24 hours before the operation. Blood samples for determination of fibrinolytic activity and factor VIII in plasma were obtained before and immediately after the end of compression and application of a stocking, respectively. Also the level of plasminogen activator activity (PA) in the vein wall was determined. Neither IPC nor the application of an elastic stocking had any demonstrable effect on the variables studied. In addition, the results did not vary with the rate (slow or quick) or with the duration of IPC, sex or site of application (arm or leg) of IPC. Blood samples from the compressed leg were also uninfluenced.

Intermittent pneumatic compression (IPC) and graduated static compression stockings have proved effective in the prevention of postoperative deep venous thrombosis (Sabri et al., 1971; Hills et al., 1972; Clark et al., 1974; Calnan & Allenby, 1975; Holford, 1976; Scurr et al., 1977). The pump cycle pattern in IPC and thereby its effect on venous flow has varied substantially between investigations on record. The preventive effect of the method does not, however, seem to vary with the pumping pattern (Ah-See et al., 1976), or with changes in the degree of static compression.

It is known that venous occlusion stimulates a local release of activators of the fibrinolytic system from the vein wall (Clarke et al., 1960; Nilsson et al., 1970a) and in 1973 Allenby et al. ascribed the prophylactic effect of IPC to the activation of the fibrinolytic system by its pumping effect.

The purpose of the present investigation was to analyse the effect of slow and quick IPC and graduated static compression on the release of plasminogen activators from the vein wall. Since it is known that not only plasminogen activator activity (PA) but also factor VIII related antigen is synthesised in the vessel wall and that both can be released by the same sort of stimuli (Mannucci et al., 1975; Nilsson et al., 1980), both factors were studied.

### MATERIAL AND METHOD

#### *Compression technique*

Two types of pumps were used, viz. Flowtron-Aire AC/200 and Flowpulse 1100 Huntleigh Medical Limited, England. The former produced slow intermittent compression with an inflation period of about two minutes to the pressure of 40 mmHg and required about two minutes to deflate, after which a new cycle was started; the latter, 3 sec and 20 sec, respectively, and the same pressure of 40 mmHg.

Two types of graduated static compression stockings were used. According to the manufacturer's description, these stockings exert pressures of respectively 18 and 35 mmHg at the ankle.

IPC was applied either to an arm or to a leg for at least the last two hours before the operation (Table I). The patients treated with graduated static compression stockings wore a stocking on one leg for 24 hours before the operation.

The limb to which IPC or elastic compression was to be



Table I. Duration and site of application of intermittent pneumatic compression (IPC)

|                     | IPC  |       |
|---------------------|------|-------|
|                     | Slow | Quick |
| Number              | 23   | 15    |
| Time of compression |      |       |
| 2-3 h               | 13   | 9     |
| >3 h                | 10   | 6     |
| Limb compressed     |      |       |
| Arm                 | 6    | 5     |
| Leg                 | 17   | 10    |

applied in a given patient was chosen at random and either the operated leg or the control leg was used for one of the two kinds of compression.

#### Patients

Twenty-three patients (9 men and 14 women) with a median age of 42 (range 29-66 years) were treated with slow IPC and 15 (5 men and 10 women) with a median age of 43 years (range 27-68) with quick IPC. Twelve patients (3 men and 9 women) with a median age of 58 years (range 24-78 years) were treated with graduated static compression, six with the lower and six with the higher pressure.

None of the patients were overweight and all were healthy, except for the varicose veins for which they were operated upon. None of the patients had a previous history of deep venous thrombosis. The operations were done under general anaesthesia and never took more than 60 minutes.

#### Laboratory methods

Blood samples from an antecubital vein were obtained before and immediately after IPC and before application of the elastic stockings the day before the operation and again after removal of the stockings just before the operation. The following determinations were made: factor VIII activity (VIII: C), factor VIII related antigen (VIII: Ag), fibrinolytic activity on unheated fibrin plates with citrated plasma and resuspended euglobulin precipitate as test solutions. The methods have been described in detail

elsewhere (Nilsson & Olow, 1962; Nilsson et al., 1970a; Holmberg & Nilsson, 1973). In 11 patients in whom an arm was subjected to IPC, the blood samples were obtained from that arm.

In four patients a catheter was placed in the femoral vein peroperatively and blood samples were obtained from the compressed leg during the actual inflation period, during the deflation period and again after the end of pumping period. In addition, a blood sample was obtained from an arm vein before and after the period of IPC. The compression pattern was slow in two patients and quick in two.

A biopsy specimen of a vein was obtained from the foot or from the back of the hand on the side treated with IPC or a stocking. Control biopsy specimens were obtained from the opposite side. The PA was assessed with Todd's histochemical method (1959), as modified by Pandolfi (Pandolfi et al., 1967; 1968; 1972). The activity is expressed in arbitrary units (normal range of hand veins is 6-11 arbitrary units).

The investigation was approved by the Ethical Committee, University of Lund, and all patients gave their written consent.

#### Statistical methods

The data were analysed with Student's paired *t*-test. The difference between the biopsy specimens were studied with Wilcoxon's rank sum test. A difference was considered significant when  $p < 0.05$ .

## RESULTS

It is clear from Table II that neither slow nor quick IPC had any demonstrable effect on VIII: C or VIII: Ag. Neither did IPC nor elastic stockings stimulate the fibrinolytic activity of the plasma as determined with the fibrin plate method.

No difference in PA was found in biopsy specimens of the vein on the side treated with IPC or with an elastic stocking, and that from the contralateral side, and, the response of the arms to IPC was the same as that of the legs (Table III).

In four patients blood samples were obtained from the compressed leg locally during the inflation

Table II. Factor VIII (%), fibrinolytic activity on unheated fibrin plate measured with citrated plasma (mm<sup>2</sup>) and resuspended euglobulin precipitate (mm<sup>2</sup>), mean and range

|                       | Intermittent pneumatic compression |              |          |              |              |          |
|-----------------------|------------------------------------|--------------|----------|--------------|--------------|----------|
|                       | Slow (n=21)                        |              |          | Quick (n=14) |              |          |
|                       | Before                             | After        | <i>p</i> | Before       | After        | <i>p</i> |
| VIII: C               | 146 (85-265)                       | 137 (68-285) | >0.30    | 130 (48-230) | 131 (60-205) | >0.95    |
| VIII: Ag              | 131 (66-244)                       | 122 (64-277) | >0.60    | 128 (75-202) | 113 (55-192) | >0.30    |
| Citratplasma          | 23 (0-92)                          | 18 (10-51)   | >0.05    | 16 (0-62)    | 21 (10-51)   | >0.05    |
| Resusp. euglob. prec. | 106 (28-261)                       | 105 (42-179) | >0.90    | 115 (0-219)  | 122 (10-186) | >0.70    |

Table III. Median and range values of plasminogen activator activity (PA) expressed in arbitrary units of compressed arm and leg

|            | Intermittent pneumatic compression |               |    |                | Graduated static compression |                |
|------------|------------------------------------|---------------|----|----------------|------------------------------|----------------|
|            | n                                  | Slow          | n  | Quick          | n                            | Median (range) |
| Arm        |                                    |               |    |                |                              |                |
| Compressed | 5                                  | 6.0 (5.5-9.0) | 5  | 6.0 (4.5-9.0)  |                              |                |
| Control    |                                    | 6.5 (6.0-9.0) |    | 6.5 (4.5-9.0)  |                              |                |
| Leg        |                                    |               |    |                |                              |                |
| Compressed | 12                                 | 5.0 (1.5-7.5) | 10 | 5.5 (3.5-8.0)  | 12                           | 7.0 (2.0-9.5)  |
| Control    |                                    | 6.0 (3.0-8.5) |    | 6.5 (3.5-10.5) |                              | 6.5 (3.5-9.0)  |

and deflation period and after the end of the compression period. Blood samples were also taken from an arm vein in these four patients before and after IPC. The values of factor VIII (VIII: C and VIIIIR: Ag) and the fibrinolytic activity on unheated fibrin plates did not vary with the phase i.e. inflation or deflation period, or the pattern of compression (slow or quick). Neither were any noteworthy changes seen after the end of the pumping period and all values were normal except in one patient where factor VIII and the fibrinolytic activity levels were subnormal. In this patient, however, the PA in a foot vein was normal in the compressed leg as well as in the control leg, viz. 8.0 and 8.5 arbitrary units.

The duration of compression, sex of the patient or the extremity compressed (leg or arm) did not influence factor VIII, fibrinolytic activity on fibrin plate or the PA in the vein wall ( $p > 0.1$ ).

#### DISCUSSION

Minor surgery has little or no effect on the fibrinolytic activity in plasma in the early postoperative course (Olow, 1963; Mansfield et al., 1972; Åberg &

Nilsson, 1978). Varicose vein surgery can be considered as minor surgery and in our material the operation was always short and the preoperative blood loss small. It is known that the PA in the wall of a varicose vein does not differ from that in a healthy vein at the same level (Pandolfi et al., 1968; Yao et al., 1974; Wolfe et al., 1979).

Several investigations (Allenby et al., 1973; Knight & Dawson, 1976; Tarnay et al., 1980) suggest that per-postoperative IPC stimulates the fibrinolytic system during and after the operation and that this activation tends to prevent postoperative deep venous thrombosis. Other investigations (Browse, 1976; O'Brien et al., 1979) argue against such an effect of IPC. No explanation can be offered for this discrepancy. Allenby et al. and Knight & Dawson as well as Tarnay et al. used only the euglobulin clot lysis time (ECLT) as an indicator of the fibrinolytic activity. ECLT is regarded as a simple, though somewhat crude screening method (Nilsson, 1975). The method is not so precise as the fibrin plate method. In addition, the latter method is independent of the fibrinogen content of the plasma.

Our results corroborate O'Brien's finding who also used fibrin plate lysis that IPC does not stimulate the plasma fibrinolytic activity. This holds also for graduated static compression stockings.

Since the fibrinolytic activity in the plasma after venous occlusion of the arms is three to four times higher than that in the legs (Pandolfi et al., 1967) also the arms were subjected to IPC. Neither did this procedure influence the results on fibrin plates.

We determined the PA in the vein wall and factor VIII because both are synthesised in venous endothelium (Nilsson et al., 1970b; Bloom et al., 1973) and are released into the bloodstream by the same

#### Graduated static compression (n=4)

| Before        | After         |
|---------------|---------------|
| 219 (195-257) | 211 (195-220) |
| 211 (144-272) | 191 (150-216) |
| -             | -             |
| 128 (67-190)  | 118 (56-165)  |

stimuli such as venous occlusion, DDAVP and adrenalin (Nilsson et al., 1980; Rijken et al., 1980). These factors have not been studied before in association with IPC and with compression by an elastic stocking. The level of factor VIII related antigen is thus an indicator of the degree of the release of this factor from the vessel wall. An increase in factor VIII might also have a thrombogenic effect. In contrast to these above mentioned stimuli neither IPC nor graduated static compression had any noteworthy stimulating effect on the release of PA or factor VIII in our investigation.

Since all the previous investigations have used only slow IPC we studied also quick IPC, which has quite a different effect on the flow pattern, but the same prophylactic effect on thrombosis (Ah-See et al., 1976). We did not find variation of the rate or the duration of IPC to have any effect on the variables studied. Neither was any variation seen with the degree of static compression applied. Robertson et al. (1972) have shown that venous occlusion does not raise the fibrinolytic activity in the general circulation, but only locally in the area compressed. However, we found no activation of fibrinolysis in blood samples obtained from the limb treated with IPC and no regular pattern in phase with the pump cycles.

The findings appear to warrant the conclusion that the thromboprophylactic effect of IPC and graduated static compression must be due to factors other than those affecting coagulation and fibrinolytic factors.

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COMPARISON OF THE PLASMINOGEN ACTIVATOR ACTIVITY IN BIOPSY SPECIMENS OF  
HUMAN ARTERIES AND VEINS

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ABSTRACT

Biopsy specimens were obtained from 161 arteries and concomitant veins in different anatomic regions in healthy persons and in patients undergoing various surgical procedures. The plasminogen activator (PA) activity was determined with a fibrin slide technique. The activity in the arteries did not differ significantly from that in the veins except in the epigastric veins, where the activity was significantly higher. The arterial PA activity was not influenced by age, sex, body-weight, uremia or diabetic disease. A correlation in activity was demonstrated between arteries and concomitant veins in all the anatomic regions studied.

Introduction

An intact fibrinolytic system prevents fibrin deposition and dissolves fibrin from the endothelial cells in the vessel wall (1). Plasminogen activators (PA) activate the fibrinolytic system. The interest in vascular PA has been focused mainly on the veins. This can partly be ascribed to the fact that the decrease in the PA activity in the veins is an important factor in the pathogenesis of venous thrombosis (2,3,4,5). Arterial PA activity has been studied less systematically. Most studies on arterial PA activity are based on postmortem specimens because it is easier to obtain biopsy specimens of human veins than in arteries. The physiological and clinical significance of an alteration in the PA activity in the arteries is not properly understood.

The PA activity in superficial veins varies from one anatomic region to another (6,7,8,9,10,11). The activity in superficial leg veins is lower than that of the corresponding deep veins obtained from the same level (11). There is a correlation in the PA activity between superficial hand and foot veins (12) and between superficial and deep veins of the leg (11). The PA activity is low in veins in certain conditions, such as obesitas (13), diabetes mellitus (14) and after surgical trauma (15,16,17).

It is not known whether the arterial PA activity varies between different anatomical regions. Neither is it known whether any correlation exists between the activity in arteries and veins at a given anatomic level. In one small series a correlation in PA activity was found between temporal arteries



and superficial hand veins in diabetics (18). Furthermore, it is not known whether any diseases are capable of influencing the arterial PA activity.

The aim of this study was to assess the PA activity in arteries and concomitant veins from different anatomic regions in healthy persons and in patients with severe arteriosclerosis, uremia or diabetes mellitus. The study was also designed to detect any correlations in activity between various arteries and veins.

#### Material and Method

In patients undergoing surgery totally 161 pairs of arterial and vein specimens were obtained from:

- \* The popliteal vessels in 23 patients undergoing below knee amputation because of severe arteriosclerosis. The arteries were occluded in all the patients but three.
- \* The external pudendal vessels in the groin in 15 patients undergoing varicose vein surgery. These patients were otherwise healthy.
- \* The inferior epigastric vessels in 10 patients undergoing surgery because of inguinal hernia. These patients were otherwise healthy. Biopsy specimens from the inferior epigastric vessels were also obtained in 52 uremic patients, undergoing renal transplantation because of diabetic nephropathy (21 patients) or other renal diseases (31 patients).
- \* The iliac vessels in 46 of the above mentioned 52 uremic patients.
- \* The renal vessels in 15 healthy kidney donors.

All biopsy specimens were obtained as soon as possible during the operation and always within two hours. Patients undergoing amputation or treatment of inguinal hernia repair were under spinal analgesia; the others, under general anaesthesia. The age and sex distribution is given in Table I, which also includes the distribution of the diabetes.

Table 1 The distribution of age, sex and diabetes

| Operation performed                         | Age        | Females | Males | Diabetes mellitus |
|---------------------------------------------|------------|---------|-------|-------------------|
| Amputation of leg                           | 71 (28-89) | 13      | 10    | 11                |
| Treatment of varicose veins                 | 56 (36-66) | 9       | 6     | -                 |
| Treatment of inguinal hernia                | 53 (30-76) | 1       | 9     | -                 |
| Kidney transplantation (epigastric vessels) | 37 (10-64) | 22      | 30    | 21                |
| (Iliac vessels)                             | 37 (10-64) | 17      | 29    | 13                |
| Nephrectomy of living donor                 | 44 (26-72) | 8       | 7     | -                 |



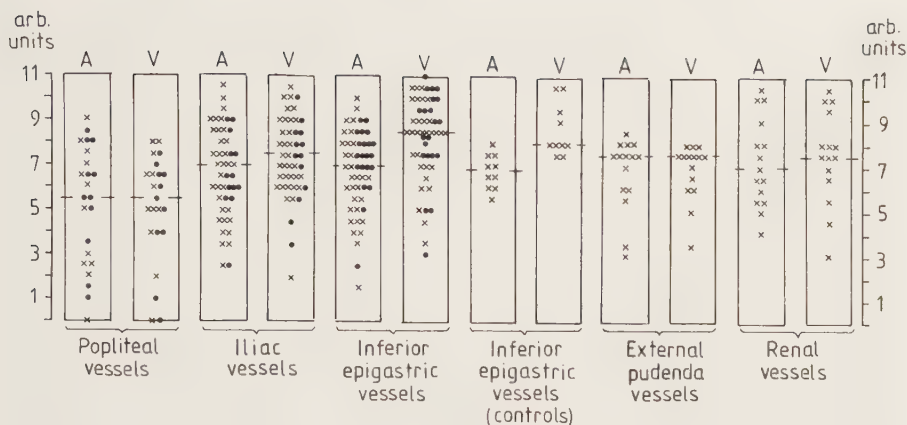


Fig. 1 The individual PA activity in arbitrary units in arteries (A) and concomitant veins (V). ● denotes diabetic patients.

PA determination. Biopsy specimens from an artery and a concomitant vein were always obtained at one and the same level. The PA activity was determined histochemically with Todd's (19) fibrin slide technique, as modified and described in detail by Pandolfi et al. (7). This method is semiquantitative and the PA activity is expressed in arbitrary units. The reproducibility of this method is good (20).

### Statistical calculations

Mann-Whitney's rank sum test and the Student's t-test were used. The correlation coefficient was calculated according to Spearman's rho ( $\rho$ ) (21). When  $p$  was  $<0.05$ , the difference was regarded as significant.

### Results

The individual PA activity in arbitrary units in arteries and veins is given in Fig. 1. The median PA was the same in arteries and concomitant veins in all regions except in the inferior epigastric vessels, where the activity was significantly higher in the veins. This difference was true both for 10 healthy subjects undergoing treatment of inguinal hernia ( $p<0.01$ ) and for 52 uremic patients ( $p<0.001$ , Fig. 1). Figures 2 and 3 show the histochemical appearance of the PA activity of a epigastric artery and concomitant vein.

Age, sex and body-weight did not influence the median PA in the various arteries in uremic patients or in patients with arteriosclerosis of the legs (Table 2). Neither did diabetes affect the median PA in the various arteries or veins examined (Fig. 1).

Median PA was lower in the popliteal arteries than in the arteries obtained from the other regions ( $p<0.05$ , Table 2). The individual PA activity in three patent popliteal arteries is given in Table 2.

A correlation in PA activity was found between arteries and concomitant veins in all the anatomic regions examined (Table 3). In uremic patients an intraindividual correlation in PA activity was also found between the epigastric artery and the iliac artery ( $\rho=0.35$ ,  $p<0.05$ ).

Table 2 Median value and range of PA activity in arbitrary units in various arteries and the distribution of age, sex and diabetes mellitus

|               | Popliteal artery |                | Epigastric artery |                | Iliac artery |                |
|---------------|------------------|----------------|-------------------|----------------|--------------|----------------|
|               | No. of pats      | Median (range) | No. of pats       | Median (range) | No. of pats  | Median (range) |
| Age <50       | 3                | *              | 39                | 7.0 (1.5-10.0) | 32           | 6.5 (2.5-10.5) |
| Age ≥50       | 20               | 5.75 (1.0-9.0) | 13                | 6.75(4.0-9.5)  | 14           | 6.5 (3.5- 9.0) |
| Female        | 13               | 5.5 (0.0-8.5)  | 22                | 7.25(3.5-9.5)  | 17           | 6.5 (2.5- 9.0) |
| Male          | 10               | 6.5 (2.0-9.0)  | 30                | 7.0 (1.5-10.0) | 29           | 6.5 (3.5-10.5) |
| Diabetics     | 11               | 5.5 (1.0-8.5)  | 21                | 7.5 (2.5-9.0)  | 13           | 6.5 (2.5-9.0)  |
| Non-diabetics | 12               | 5.0 (0.0-9.0)  | 31                | 6.5 (1.5-10.0) | 33           | 6.5 (2.5-10.5) |

\*) The individual PA activity in arb. units (patent arteries): 0.0, 2.0 and 6.0

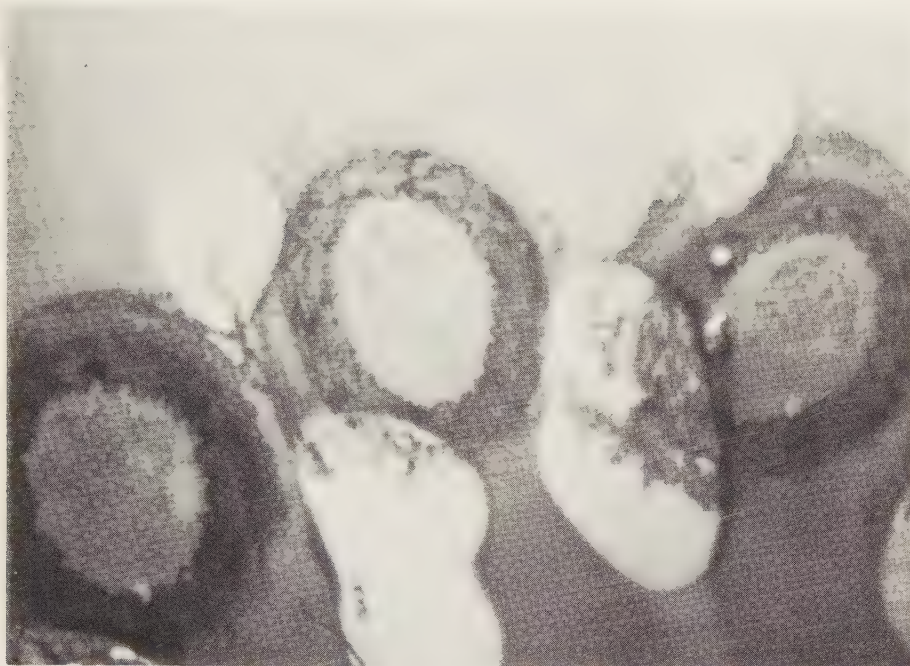


Fig. 2 Cross sections of the epigastric artery with an incubation time of 20 min tested with the fibrin slide technique. White area denotes the PA activity, localized to the vasa vasorum of the adventitia. Mag. x 10.

Table 3 Correlation coefficient ( $\rho$ ) between various arteries and concomitant veins and p values

|                           | No. of pats | $\rho$ | p     |
|---------------------------|-------------|--------|-------|
| Popliteal vessels         | 23          | 0.42   | <0.05 |
| Iliac vessels             | 46          | 0.38   | <0.05 |
| Inf. epigastric vessels   |             |        |       |
| (uremic patients)         | 52          | 0.44   | <0.01 |
| (healthy persons)         | 10          | 0.65   | <0.05 |
| External pudendal vessels | 15          | 0.60   | <0.05 |
| Renal vessels             | 15          | 0.73   | <0.01 |



Fig. 3

Cross sections of the epigastric vein with an incubation time of 20 min tested with the fibrin slide technique. Mag. x 10.

## Discussion

Most published data on PA activity in arteries are based on specimens obtained from cadavers. Little or nothing is known as to whether or how the PA activity is influenced by the terminal disease, anoxia, chock, interval between death and autopsy and the storage of the cadaver. This makes it difficult to compare the findings with those made in our study, in which the specimens were obtained from patients undergoing surgery.

In cadavers Coccheri and Astrup (22), who used an extraction technique, and Todd (23, 24), who used a histochemical method, always found the PA activity in the adventitia of large veins to be higher than that in the adventitia of arteries. In this study the activity in the arteries was the same as in the concomitant veins, which is in agreement with Pandolfi's (25) findings in retinal vessels in cadavers. One exception in our study was, however, that the activity in the epigastric veins was significantly higher than that of the corresponding arteries both in healthy persons and in uremic patients. It is not known why there was a difference in the activity between arteries and veins in this region, but not in the others.

Postmortem studies of the PA activity in arteries for any variation with age have given contradictory results (26,27,28,29). Judging from our study, age does not influence the activity in the arteries. Neither was it found to vary with sex, which corroborates the findings by Lieberman and Kellogg (27) and Donner et al. (29).

In uremic patients the fibrinolytic activity in the blood is low. In these patients there is a tendency to deposition of fibrin, which has been ascribed to a marked increase in inhibitors of plasminogen activators (30). From this study it can be concluded that the fibrin deposition is not due to the lowness of the PA content of the arteries in uremic patients.

The PA activity in arteries and veins in patients with frequently severe and long-standing uremia due to diabetes mellitus did not differ from that in the corresponding vessels in patients with uremia due to other renal diseases. The activity found by Almér et al. (18) in the temporal arteries in diabetics, did not differ from that in our study. No further data on the activity in human arterial specimens from diabetics are available. It would thus appear that diabetes per se does not influence the PA activity in the arteries and deep veins in the various regions examined in the present investigation. In superficial hand veins, the PA activity is lower in about one fourth of all diabetics (14).

The finding of the same arterial activity in all anatomic regions above the knee corroborate the findings by Donner et al. (29) in cadavers. As seen from Fig. 1, the range of variation in PA activity of the arteries and concomitant veins in healthy persons (epigastric, pudendal and renal vessels) is narrower than that of vessels obtained from patients with severe arteriosclerosis or uremia. The significantly lower PA activity in the popliteal arteries can be explained by the fact that these arteries were obtained from patients undergoing amputation because of severe arteriosclerosis. Furthermore all arteries except three were occluded. The explanation is strengthened by the findings of a low PA activity in specimens of arteriosclerotic aortae obtained from patients undergoing reconstructive surgery of the aorta and/or iliac arteries (Ljungnér et al., unpublished observation). However, data published by researchers in arteriosclerosis are conflicting, some arguing for a decreased activity (28, 31); others for an increased activity (29, 32). The pathogenetic role of the fibrinolytic activity in arteriosclerosis is debatable and our study did not shed further light on this problem.

In conclusion, the activity in arteries and concomitant veins did not differ except in the epigastric vessels, where the activity was higher in the veins in healthy persons as well as in uremic patients. Age, sex, bodyweight, uremia and diabetic disease did not influence the arterial PA activity. A correlation was found between arteries and veins in all regions examined.



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